

Apoptotic efficiency of capecitabine and 5-fluorouracil on human cancer cells through TRPV1 channels

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Received 25 August 2019; revised 20 September 2019

The expression of transient receptor potential protein channels increases intensively colon and breast cancer cells. We aimed to reveal the role of 5-fluorouracil (5FU) and capecitabine along with Transient Receptor Potential Vanilloid 1 (TRPV1) channels in breast and colon cancer cells. Breast (MCF-7) and colon (Caco-2) cells were cultured and the study was planned as 7 main groups. Cells in the group were incubated with 5FU and capecitabine for 24 h and then incubated with TRPV1 channel antagonist capsazepine and stimulator capsaicin. The effects of medicines were investigated on molecular pathways of apoptosis. It was concluded that the administration of TRPV1 channel stimulator capsaicin in both cancer cells significantly increased the degree of intracellular Ca^{2+} levels and apoptosis levels compared to the control group whereas, the use of TRPV1 channel inhibitor capsazepine, significantly decreased the degree of apoptosis levels. The apoptotic effects of 5FU and capecitabine on both colon and breast cancer cells are directly related to TRPV1 channels and TRPV1 channels play an important role in the apoptosis. The apoptotic cell lines activity of capecitabine was higher in breast cancer cells, while that of 5FU was more pronounced in colon cancer cells.

Keywords: Apoptosis, Breast and colorectal cancers, Capsaicin, Capsazepine, Chemotherapy, Osteosarcoma

Breast and colon cancers are common and are among the significant causes of death worldwide. Breast cancer is the most common type of cancer among all cancers and is among the leading causes of cancer-related deaths in women¹. Colon cancer is the third most common cancer in men, following lung and prostate cancers, and the second most common cancer in women, after breast cancer². Chemotherapy in breast and colon cancer is often used in the preoperative and postoperative periods. Significant progress has been made in the treatment with the development of chemotherapy strategies that are applied depending on the various cancer subtypes and stages as a result of recent studies, especially breast cancer³.

5-Fluorouracil (5FU) is one of the chemotherapeutic agents used in both colon and breast cancer. The most well-known antitumor effect of 5FU is to bind DNA and RNA and thereby to block DNA synthesis and cell proliferation. 5FU is widely used in head, neck, breast cancers and aerodigestive tract cancers with a broad spectrum of activity against solid tumors, alone or in combination chemotherapy⁴.

Capecitabine is a prodrug used for treating metastatic and locally advanced breast cancers that do not respond to anthracycline or paclitaxel treatment. It is administered orally and metabolized and converted to 5FU, the active agent, in the liver⁵. Capecitabine is also used in the treatment of adjuvant and especially metastatic colon cancer and metastatic gastric cancer⁶.

There are 6 subfamilies of mammalian TRP channels: TRPC (canonical), TRPV (vanilloid), TRPM (melastatin), TRPP (polycystin), TRPML (mucolipin) and TRPA (ankyrin)⁷. TRP channels are sensitive to stimuli such as temperature, pH, injury, cations, cytokines and mechanical stress⁸. Besides, TRP channels respond fairly well to oxidative stress mediators including reactive oxygen (ROS) and reactive nitrogen species (RNS) and other electrophiles⁹.

The TRP protein family contains different groups with calcium ion (Ca^{2+}) permeable and non-selective cation channels and is found in many living organisms. Intracellular Ca^{2+} plays an important role in oxidative stress and apoptosis as well as inactivation of TRP channels by receptor stimulation and enzymes such as Ca^{2+} dependent protein phosphatase¹⁰. It is known that chemotherapeutic

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agents increase free oxygen radicals and intracellular Ca^{2+} levels in the cell and lead to oxidative stress. TRP channels have been reported to play an important role in the development of numerous diseases and one of these involves TRPV1 channels¹¹. It has been reported that stimulation of TRPV1 channels induces apoptosis in many cancer cells such as osteosarcoma, colon, glioma, and pancreas, inhibiting the growth of cancer cells while preserving normal cells undamaged^{12,13}. Similar effects of TRPV1 channels have been also demonstrated in breast cancer cells¹⁴.

In this study, we administered 5FU and capecitabine agents to colon and breast cell cancer cell cultures and investigated the role of TRPV1 channels, which are known to be abundant in breast and colon cancer cells, and sensitive to oxidative stress. We also aimed at revealing the role of these drugs on TRPV1 channels and intracellular Ca^{2+} values.

Materials and Methods

Cell culture, reagents, and dyes

Human Breast Cancer (MCF-7) and Colon Cancer (Caco-2) cell lines were purchased from the Culture Collection of Animal Cells, Foot and Mouth Disease (ŞAP) Institute, Ankara, Turkey. MCF-7 and Caco-2 cells were cultured in Roswell Park Memorial Institute-1640 (RPMI-1640) and Dulbecco's modified Eagle's medium (DMEM) respectively. All mediums containing 10% fetal bovine serum (FBS) (Fisher Scientific, and 1% penicillin/streptomycin (Thermo-Fischer). Cells were evenly distributed as 1×10^6 cells in each of 8-10 flasks (filter cap, sterile, 5 mL, 25 cm²). Cells were incubated at 37°C at 5% CO₂ in a humidified incubator. After cells have reached 75–85% confluence, cell was incubated with the chemical compounds described in the groups section. Cells were examined daily for evidence of contamination. After treatments, the cells were detached with % 0.25 Trypsin–EDTA for analysis and split into the sterile falcon tubes for analyses.

DMEM, RPMI 1640, Trypsin–EDTA, Fetal Bovine Serum, and penicillin-streptomycin and Dimethyl sulfoxide, Dihydrorhodamine-123 (DHR 123) were obtained from Sigma Aldrich (St. Louis, MO), Fura 2 (AM) calcium fluorescent dye was purchased from Calbiochem (Darmstadt, Germany). A mitochondrial stain 5,50, 6,60-tetrachloro-1,10,3,30-tetraethyl benzimidazolyl carbocyanine iodide (JC-1)

and Probenecid were obtained from Santa Cruz (Dallas, Texas, USA). Pluronic® F-127 was obtained from Biovision (San Francisco, USA). Caspase-3 (AC-DEVD-AMC) and Caspase 9 (AC-LEHD-AMC) substrates were obtained from Enzo (Lausen, Switzerland). APOPercentage assay with releasing buffer was purchased from Biocolor (Belfast, Northern Ireland).

Study Groups

The study was planned as 7 main groups below:

- Group 1 (Control): None of the study drugs were used and were kept in a flask containing the same cell culture condition.
- Group 2 (Capec): Cells were incubated with 2 μM Capecitabine for 24 h¹⁵.
- Group 3 (Capec+Czpn): Cells were incubated with 2 μM Capecitabine for 24 h and then incubated with TRPV1 channel antagonist Capsazepin (Czpn, 0.1 mM, 30 min).
- Group 4 (Capec+5FU): Cells were incubated with 2 μM Capecitabine and 2,5 μM 5-Fluorouracil for 24 h.
- Group 5 (Capec+5FU+Czpn): Cells were incubated with 2 μM Capecitabine and 2,5 μM 5-Fluorouracil for 24 h and then incubated with TRPV1 channel antagonist Capsazepin (Czpn, 0.1 mM, 30 min).
- Group 6 (5FU): Cells were incubated with 2,5 μM 5-Fluorouracil for 24 h¹⁶.
- Group 7 (5FU+Czpn): Cells were incubated with 2,5 μM 5-Fluorouracil for 24 h and then incubated with TRPV1 channel antagonist Capsazepin (Czpn, 0.1 mM, 30 min).

For Apoptosis, Intracellular Reactive Oxygene Species, Mitochondrial depolarisation, Caspase 3 and Caspase9 experiments, the cells were further treated with TRPV1 channel agonist Capsaicin (Cpsin, 0.1 mM, 10 min) for activation of TRPV1 channel before related analysis. During calcium signaling analysis (Fura-2/AM), cells were stimulated on 20th cycles with 0.1 mM Cpsin in the existence of 1.2 mM Calcium and calcium free buffer in extracellular environment.

Measurement of intracellular free calcium concentration ($[\text{Ca}^{2+}]_i$)

Intracellular free calcium concentration ($[\text{Ca}^{2+}]_i$) was measured with UV light excitable Fura 2 acetoxymethyl ester as an intracellular free Ca^{2+} indicator. After cell culture treatments, MCF-7 and Caco-2 cells were incubated with HEPES-buffered

saline [HBS; 5 mM KCl, 145 mM NaCl, 10 mM D-glucose, 1 mM MgCl₂, 1.2 mM CaCl₂, 10 mM HEPES and 0.1% (w/v) BSA]; pH 7.4] containing 5 μM fura-2 AM and 0.05% (w/v) Pluronic F-127 for 1 h at 37°C in the dark. The loaded cancer cells were washed twice with HBS and covered with 1000 μL of HBS supplemented with 2.5 mM probenecid for at least 20 min at 37°C in the dark to allow for Fura-2 AM de-esterification. Cells were seeded in clear flat-bottom black 96-well culture trays (Grainer Cell Star, Life Sciences USA) at a density of 4 × 10⁴ cells/per well. Fluorescence emission intensity at 510 nm was determined in individual wells using a plate reader equipped with an automated injection system (SynergyTM H1, Biotek, USA) at alternating excitation wavelengths of 340 and 380 nm every 3 s for 50 acquisition cycles (cycle: 3 s; gain: 120) in response to agonists (Cpsin, 0.1 mM) added with the automated injector. [Ca²⁺]_i in cells was expressed as the average emission at 510 nm in individual wells in response to excitation at 340 nm (Ca²⁺ bound)/380 nm (Ca²⁺-free Fura 2 AM) normalized to initial fluorescence emission obtained during the first 20 cycles. Measurement of [Ca²⁺]_i including staining process modification was performed according to the method of Martinez *et al*¹⁷.

Intracellular ROS production measurement

The cells (10⁶ cells/mL for per group) were incubated with 20 μM DHR 123 as fluorescent oxidant dye at 37°C for 25 min¹⁸. The Rh123 fluorescence intensities were determined by using an automatic microplate reader (SynergyTM H1, Biotek, USA). Excitation and emission wavelengths of the analyses were 488 nm and 543 nm, respectively. We presented the data as a fold increase over the level before treatment.

Assay for Apoptosis level, caspase 3 and caspase 9 activities

The APOPercentageTM which is used as an assay for the detection and quantification of apoptosis (Biocolor Ltd., Belfast, Northern Ireland) was performed according to the manufacturer's instruction. APO Percentage dye is actively bound to phosphatidylserine lipids and transferred into the cells and apoptotic cells are stained red. The apoptosis analysis procedure was performed according to the manufacturer's instruction and Özdemir *et al*¹⁹. The cells were analyzed for apoptotic cell detection by spectrophotometry (multiplate reader) at 550 nm (SynergyTM H1, Biotek, USA).

The methods of Caspase 3 and Caspase 9 activity were based on previously reported^{20,21}. Caspase 3 (AC-DEVD-AMC) and Caspase 9 (AC-LEHD-AMC) substrates cleavages were calculated with SynergyTM H1 microplate reader (Biotek, USA) with 360 nm and 460 nm wavelengths (excitation/emission). The values were evaluated as fluorescent units/mg protein and shown as fold increase over the level before treatment (experimental/control).

Mitochondrial membrane potential (JC-1) analyses

The quantification of mitochondrial membrane depolarization was carried out by measuring the fluorescence intensity of the JC-1 a cationic dye which was measured by a single excitation wavelength of 485 nm (green) and the emission wavelength of 535 nm, the red signal at the 540 nm (excitation) and 590 nm (emission) the wavelengths (SynergyTM H1, Biotek, USA)²². Data are presented as emission ratios (590/535). Mitochondrial membrane potential changes were quantified as the integral of the decrease in the JC-1 fluorescence ratio of experimental/control.

Statistical analyses

All results were expressed as means ± standard deviation (SD). Significant values in the groups were assessed with one-way ANOVA. Statistical analyses were calculated using GraphPadPrism version 7.04 for Windows (GraphPad Software, San Diego California, the USA). *P* < 0.05 was considered significant.

Results

Effects of extracellular capecitabine and 5FU treatments on Cpsin-induced [Ca²⁺]_i concentration through TRPV1 channels activation in the MCF-7 and Caco-2 cells

As a result of administration of 5FU and capecitabine separately and together on breast cancer cell culture (MCF-7) and colon cancer cell culture (Caco-2), these chemotherapeutic agents increased the intracellular calcium levels by stimulation of TRPV1 channels in both cancer cells statistically significantly compared to the control group (*P* < 0.001 for MCF-7 and Caco-2 cells). It was found that intracellular calcium levels were significantly increased by administration of TRPV1 channel stimulator capsaicin (Cpsin) in both cancer cells, more prominently in the combination of 5FU/capecitabine compared to the control group (*P* < 0.001). On the other hand, intracellular calcium levels decreased at a significant

level with the use of the TRPV1 channel inhibitor capsazepine (Czpn) ($P < 0.001$). These effects of combined use of 5FU and capecitabine were also found to be statistically significantly higher than a single use of these agents (stimulation: $P < 0.05$, inhibition: $P < 0.001$). It was also found that 5FU significantly increased intracellular Ca^{2+} levels in both cancer cells statistically compared to capecitabine ($P < 0.001$) (Fig. 1)

Results of apoptosis and intracellular ROS production values in MCF-7 and Caco-2 Cells

Examination of apoptosis and intracellular Reactive Oxygen Species (ROS) levels with use of 5FU and capecitabine separately and together revealed that these chemotherapeutic agents led to significantly increased apoptosis and intracellular ROS levels in both cancer cells with use of TRPV1 channel stimulator (Cpsin) in the colon cancer than in the control group ($P < 0.05$) and TRPV1 channel inhibitor (Czpn) markedly decreased apoptosis and intracellular ROS levels ($P < 0.001$). This effect was found to be statistically

significantly higher in the 5FU/capecitabine combination group than in the single-agent group ($P < 0.05$). It was also seen that ROS levels in the breast cancer cells were significantly higher in the capecitabine-treated group compared to the 5FU applied group, whereas in the colon cancer cells, they were significantly higher in the 5FU group ($P < 0.001$). A comparison of the two drugs in terms of apoptosis level revealed that apoptosis levels in the 5FU group of breast cancer cells were lower ($P < 0.05$) than that of capecitabine, whereas apoptosis levels in colon cancer cells were found to be statistically higher in 5FU group ($P < 0.001$). (Fig. 2)

Results of Mitochondrial Membrane Depolarization (JC-1) and Caspase 3 and Caspase 9 levels in MCF-7 and Caco-2 Cells

As a result of examination of mitochondrial depolarization, Caspase 3 and Caspase 9 levels by administration of application of 5FU and capecitabine, it was found that these

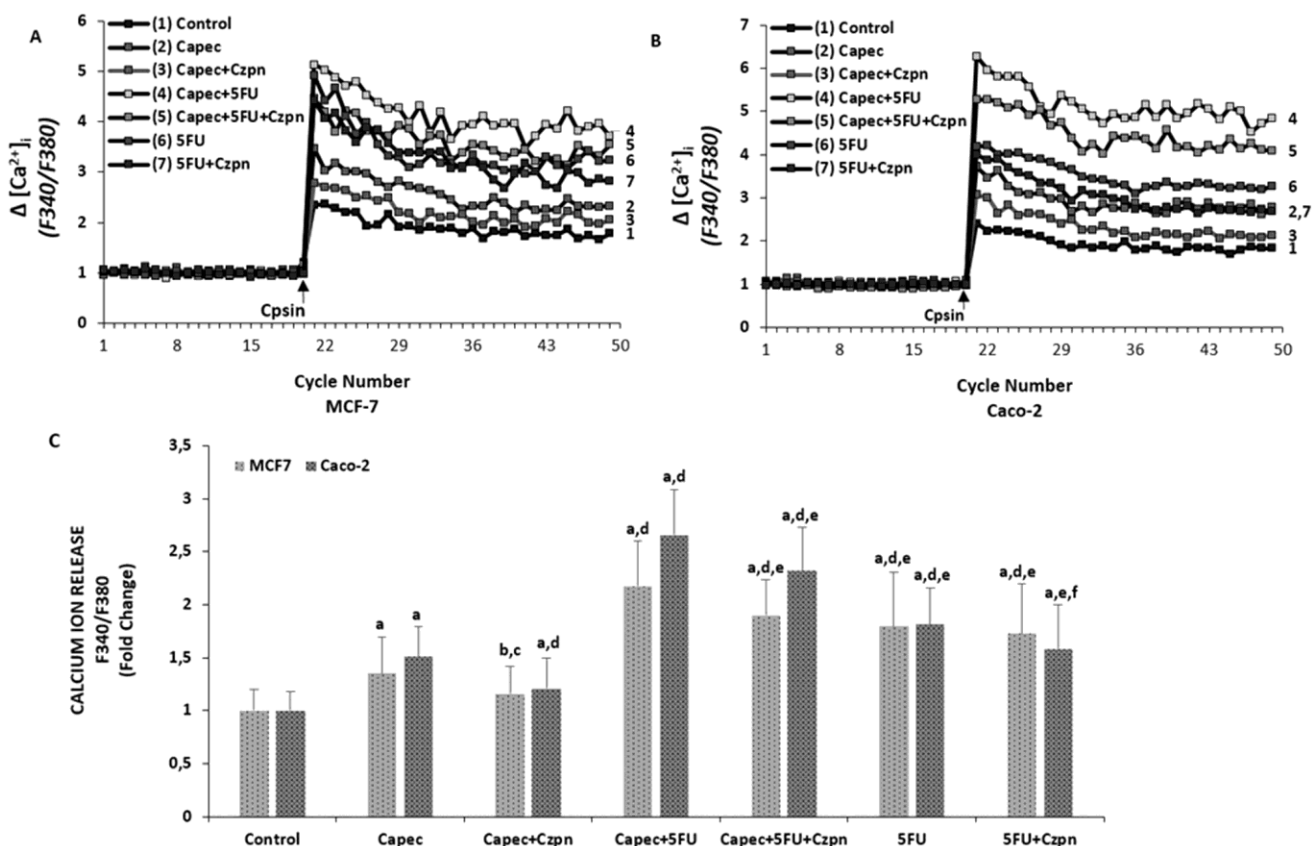


Fig. 1 — *In vitro* effect of Capecitabine (2 μ M, 24 h) and 5FU (2,5 μ M, 24 h) on the free intracellular calcium increase ($[Ca^{2+}]_i$) through TRPV1 channels in MCF-7 (A) and Caco-2 (B) cells and cellular calcium ion release (C). (n=3 and mean \pm SD). The cells are stimulated by capsaicin (Cpsin and 0.1 mM on the 20th cycle) but they were inhibited by capsazepine (Czpn and 0.1 mM for 30 min). ^a $P < 0.001$ and ^b $P < 0.05$ vs control, ^c $P < 0.05$, and ^d $P < 0.001$ vs Capec, ^e $P < 0.001$ vs Capec+5FU, ^f $P < 0.001$ vs 5FU

chemotherapeutic agents increased mitochondrial depolarization, Caspase 3 and Caspase 9 levels in both cancer cells with application of TRPV1 channel stimulator (Cpsin), being more prominent in colon cancer ($P < 0.05$) and TRPV1 channel inhibitor (Czpn) showed a significant decrease in mitochondrial depolarization, Caspase 3 and Caspase 9 levels ($P < 0.001$). These effects were found to be statistically significantly higher in the 5FU/capecitabine combination group than in the single-use group. ($P < 0.05$ for mitochondrial depolarization, $P < 0.001$ for caspase 3 and caspase 9). It was also found that capecitabine significantly increased mitochondrial depolarization levels in both

cancer cells statistically significantly compared to 5FU ($P < 0.05$). When these two drugs were compared in terms of caspase 3 and caspase 9 levels, caspase 3 levels were lower in the 5FU group compared to capecitabine in both cancer cells ($P < 0.001$) and caspase 9 levels were lower in the 5FU group than capecitabine in breast cancer cells, while caspase 9 levels were found to be statistically significantly higher in the 5FU group than in the capecitabine group in colon cancer cells ($P < 0.001$) (Fig. 3). Table 1 shows a comparison of cytosolic calcium, ROS, caspase 9, caspase 3, mitochondrial depolarization and apoptosis levels in study groups. And (Table 2) shows the effects of 5FU and

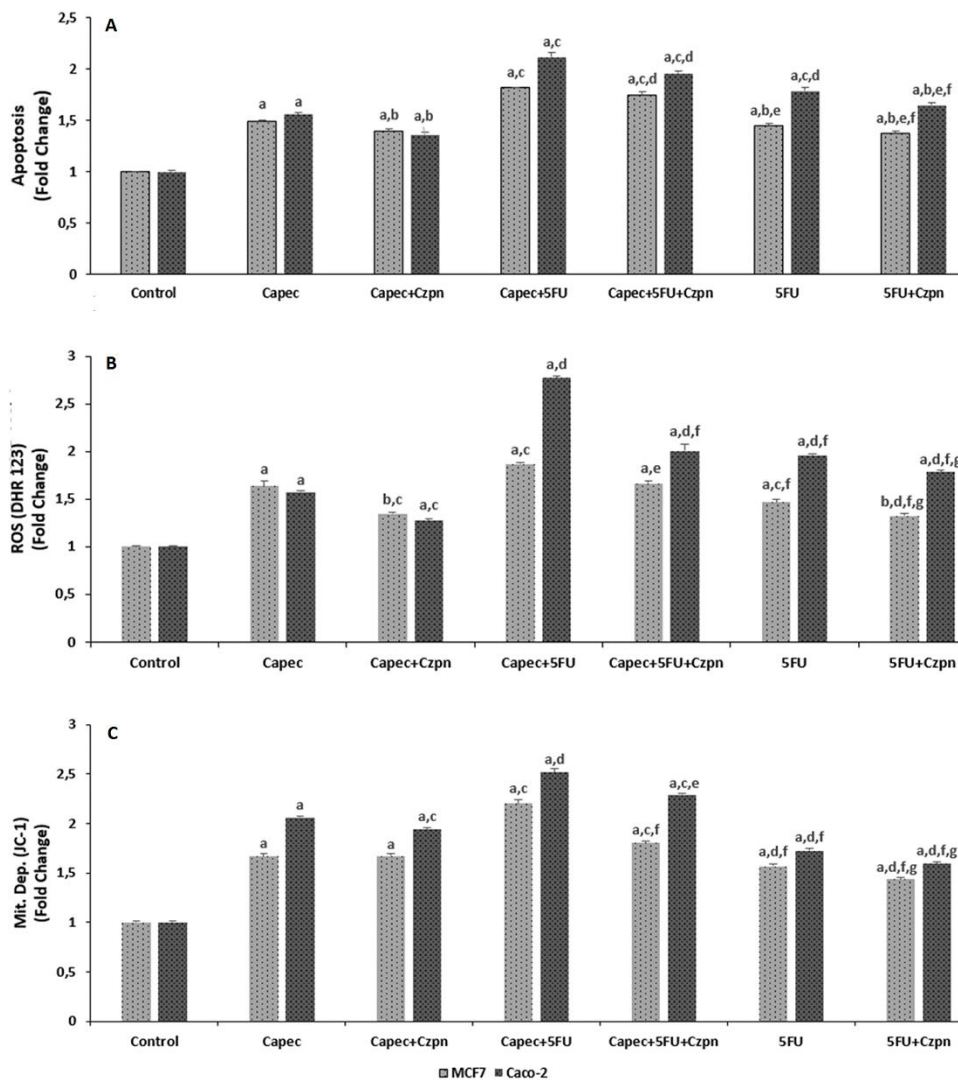


Fig. 2 — The effect of Capecitabine (2 μ M, 24 h) and 5FU (2.5 μ M, 24 h) on (A) Apoptosis levels; (B) Reactive Oxygen Species; and (C) Mitochondrial Depolarization levels in the MCF-7 and Caco2 cells (mean \pm SD and n=10). ^a $P < 0.001$ vs control, ^b $P < 0.05$ and ^c $P < 0.001$ vs Capec, ^d $P < 0.05$ and ^e $P < 0.001$ vs Capec+5FU and ^f $P < 0.05$ vs 5FU and ^g $P < 0.05$ vs 5FU

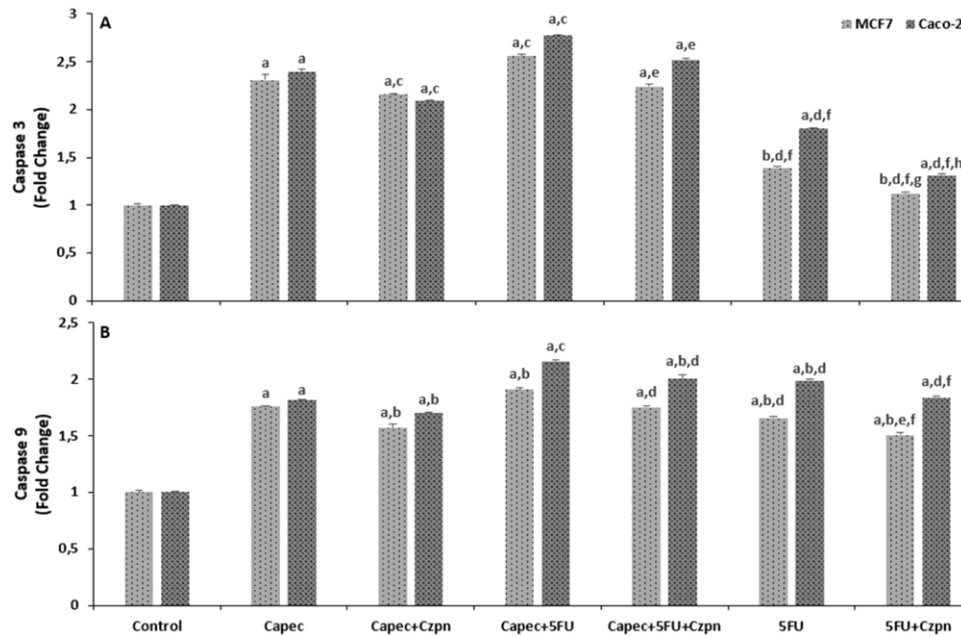


Fig. 3 — The effect of Capecitabine (2 μ M, 24 h) and 5FU (2,5 μ M, 24 h) on Caspase 3 (A) and Caspase 9 (B) levels in the MCF-7 and Caco2 cells (mean \pm SD and n=10). ^aP <0.001 and ^bP <0.05 vs control, ^cP <0.05 and ^dP <0.001 vsCapec, ^eP <0.05 and ^fP <0.001 vs Capec+5FU, ^gP <0.05 and ^hP <0.001 vs 5FU

Table 1 — Comparison of cytosolic calcium, ROS, caspase 9, caspase 3, mitochondrial depolarization and apoptosis levels with standard deviation in study groups

	Calcium Signaling	ROS	Mitoc. Dep.	Caspase 3	Caspase 9	Apoptosis
Control						
MCF-7	1 \pm 0,202	1 \pm 0,007	1 \pm 0,015	1 \pm 0,011	1 \pm 0,016	1 \pm 0,008
Caco-2	1 \pm 0,181	1 \pm 0,015	1 \pm 0,010	1 \pm 0,02	1 \pm 0,012	1 \pm 0,016
Capec						
MCF-7	1,352 \pm 0,336	1,635 \pm 0,058	1,667 \pm 0,027	2,306 \pm 0,057	1,759 \pm 0,007	1,495 \pm 0,011
Caco-2	1,509 \pm 0,287	1,574 \pm 0,019	2,059 \pm 0,020	2,394 \pm 0,029	1,819 \pm 0,005	1,559 \pm 0,020
Capec+Czpn						
MCF-7	1,159 \pm 0,262	1,349 \pm 0,018	1,673 \pm 0,027	2,154 \pm 0,009	1,57 \pm 0,037	1,394 \pm 0,028
Caco-2	1,209 \pm 0,286	1,275 \pm 0,0016	1,94 \pm 0,017	2,09 \pm 0,09	1,7 \pm 0,012	1,361 \pm 0,019
Capec+5FU						
MCF-7	2,175 \pm 0,426	1,872 \pm 0,010	2,201 \pm 0,046	2,558 \pm 0,019	1,908 \pm 0,018	1,817 \pm 0,005
Caco-2	2,661 \pm 0,424	2,772 \pm 0,014	2,517 \pm 0,037	2,779 \pm 0,005	2,159 \pm 0,013	2,116 \pm 0,049
Capec+5FU+Czpn						
MCF-7	1,896 \pm 0,339	1,664 \pm 0,023	1,806 \pm 0,019	2,243 \pm 0,018	1,753 \pm 0,014	1,748 \pm 0,033
Caco-2	2,316 \pm 0,415	2,006 \pm 0,067	2,29 \pm 0,016	2,519 \pm 0,020	2,004 \pm 0,031	1,958 \pm 0,021
5FU						
MCF-7	1,795 \pm 0,512	1,469 \pm 0,035	1,568 \pm 0,021	1,389 \pm 0,015	1,657 \pm 0,018	1,445 \pm 0,022
Caco-2	1,815 \pm 0,342	1,958 \pm 0,017	1,726 \pm 0,019	1,797 \pm 0,010	1,988 \pm 0,015	1,785 \pm 0,032
5FU+Czpn						
MCF-7	1,724 \pm 0,470	1,325 \pm 0,023	1,439 \pm 0,016	1,125 \pm 0,015	1,508 \pm 0,017	1,369 \pm 0,024
Caco-2	1,581 \pm 0,422	1,786 \pm 0,017	1,594 \pm 0,014	1,311 \pm 0,013	1,841 \pm 0,013	1,648 \pm 0,025

Table 2 — The apoptotic efficiency of 5FU and capecitabine on breast and colon cancer cells

BREAST (MCF-7)			COLON (Caco-2)		P
5FU	Capecitabine		5FU	Capecitabine	
High		Ca ²⁺ concentration	High		<0.001
	High	Intracellular ROS	High		<0.001
	High	Mitochondrial Depolarisation		High	<0.05
	High	Caspase 3		High	<0.001
	High	Caspase 9	High		<0.001
	High	Apoptosis	High		<0.05/<0.001

capecitabine on the molecular pathway of apoptosis in breast and colon cancer cells.

Discussion

Breast and colon cancers are the most common cancers in the world. In the treatment of both cancers, various chemotherapy protocols are widely used in preoperative and postoperative periods. 5FU and capecitabine, are the two most commonly used chemotherapeutic agents in colon and breast cancers^{4,5}. Capecitabine is a chemotherapy agent that is precursor of 5FU and used orally. Oral use of cabecitabine has important advantages such as ease of use, lack of complications that may develop due to intravenous drug administration, positive effects on patient's quality of life, and pharmacoeconomic reasons (*i.e.* drug cost, no need for hospitalization, *etc.*). However oral use has some disadvantages such as the unclarity of drug- taking by the patients inappropriate dosage and period and difficult controllability of this condition⁶. It has been reported that 5FU induces apoptosis in cancer cells by increasing the amount of intracellular ROS, this effect is dose and time. As a result of increasing intracellular ROS levels, damage some cellular macromolecules, depletion of endogenous antioxidants and oxidative stress begins which results in irreversible changes in components such as intracellular lipids, proteins, nucleic acids^{23,24}.

Several studies revealed the effects of subtypes of TRP channels on many different cancer cells and the relationship between the survival and the expression of TRP channels in these cancers have been demonstrated²⁵. Furthermore, many recent studies have shown abnormal TRP channel expression in different types of cancer, and TRP channels have been reported to have effects on invasion, proliferation, differentiation, and tumor vascularization in cancer cells¹³. There are also studies conducted on the relationship between TRP channels colorectal²⁶ and

breast cancer. TRPC1, TRPM2, TRPM7, TRPV2, TRPV4, and TRPV6 channels were shown to be present normal mammary tissue and their overexpression in breast cancer cells was reported^{13,27}. In a study of patients with colorectal cancer, it was reported that mRNA expression levels in TRPV3, TRPV4, TRPV5, TRPM4 and TRPC6 genes in cancer cells were lower than in normal cells²⁸. TRPV1 channels have also been found to be present in different numbers of breast cancer cell subtypes compared to normal cells and TRPV1 channel activation in breast cancer cells was reported to play an important role in all stages of the molecular pathway of apoptosis by increasing intracellular Ca²⁺ levels^{14,29}. TRPV1 channels were used in this study because it has been shown that these channels commonly expressed in both colon and breast cancers and play a role in tumor proliferation in both cancers^{12,30}.

It is known that intracellular Ca²⁺ levels have a significant impact on cancer cells and TRP channels, and they have important roles in intracellular oxidative stress and apoptosis. Ca²⁺ channels play an important role in cell proliferation, migration, apoptosis and differentiation³¹. It has been shown that all TRP channels are cation permeable and alter intracellular calcium concentrations and they have an influence on the regulation of Ca²⁺ release in numerous cell organelles³². The increase of intracellular calcium level leads to an increase of ROS amount, mitochondrial membrane depolarization and apoptosis in cancer cells. Elevation of intracellular ROS triggered oxidative stress and can cause dangerous process such as oxidative modification of intracellular nucleic acids and proteins³³. Intracellular Ca²⁺ concentration is variable and has been reported to increase in cases such as increased proliferation and abnormal differentiation which are indicative of cancer invasion¹¹.

In our study, 5FU and capecitabine were administered separately and in combination with

breast cancer cell culture (MCF-7) and colon cancer cell culture (Caco-2). Then the effects of these chemotherapeutic agents on TRPV1 channels and the effects of these channels on apoptosis were investigated. A specific stimulator (Cpsin) and inhibitor (Czpn) for TRPV1 channels were administered and the intracellular Ca^{2+} level, mitochondrial depolarization, caspase 3 and 9 values and intracellular ROS levels, all of which are intermediate stages of apoptosis of cancer cells, as well as degree of apoptosis were investigated and results were compared with the control group. As a result of the analysis, it was found that the use of both drugs resulted in TRPV1 channel activation and that there was a significant increase in intracellular calcium levels, mitochondrial depolarization and intracellular ROS levels mediated by TRPV1 channels. In the 5FU, 5FU+capecitabine groups oxidative stress-induced apoptosis levels were significantly increased compared to the control group. In conclusion, TRPV1 channel activation resulted in a statistically significant increase in intracellular calcium levels resulting in higher mitochondrial depolarization and intracellular ROS levels and degree of apoptosis in compared to the control group. As a result of TRPV1 channel inhibition, these effects were significantly reduced and apoptosis levels were found to be statistically significantly decreased compared to the control group.

Also, as result of this study, when the apoptotic effects of 5FU and capecitabine were compared, it was found that capecitabine had a statistically significantly higher effect than 5FU on breast cancer cells which are related to the molecular pathways of apoptosis by elevating intracellular levels of increasing mitochondrial depolarization and caspases 3 levels. 5FU has been shown to have a statistically significant higher level of efficacy on intracellular ROS, caspase 9, and apoptosis levels in colon cancer cells and a statistically significant higher level of intracellular Ca^{2+} concentration in both cancer cells than the capecitabine (Table 1).

Although there are studies in the literature investigating the relation and role of TRP channels in different types of cancers, a limited number of studies have examined the effects of chemotherapeutic agents on TRP channels in colon and breast cancers. It has been reported that the distal gastrointestinal tract is intensely sensitive to TRPV1 sensory neurons, extrinsically and primarily³⁴. Accordingly, it has been

reported that neuropeptides released by triggering TRPV1 channels increase neurogenic inflammation and may play a role in the development of colorectal cancer³⁰. It has also been found that TRPV1 channels have immunoregulatory roles in the intestinal mucosa due to their effects on inflammatory cells and consequently alter the tumor micro-environment³⁴. In a study of the effects of chemotherapeutic agents on TRP channels, it was shown that TRPC5 plays an important role in 5FU chemoresistant colon cells³². In another study about the impact of 5FU on breast cancer cell culture on TRP channels and cell death, similar to our study, it was demonstrated that 5FU has similar effects on all stages of the molecular pathway of apoptosis by increasing intracellular Ca^{2+} levels by TRPV1 channel activation²⁹.

Conclusion

The results of this study suggest that the apoptotic effects of 5FU and capecitabine, agents frequently used for colon and breast cancer, on tumor cells are directly related to the TRPV1 channels and that these channels play an important role in all molecular pathways of apoptosis by elevating intracellular levels of Ca^{2+} . TRPV1 channels have been shown to play crucial roles in tumor proliferation and development and exert significant effects on oxidative stress and cell death in tumor cells during chemotherapy. Further, the apoptotic activity of capecitabine, a precursor of 5FU, was observed to be higher in breast cancer cells, while that of 5FU was more pronounced in colon cancer cells.

Conflict of Interest

All authors declare no conflict of interest.

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