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Tropisetron-Induced Apoptosis: A Study on SKOV3 Cell Viability and Gene Expression

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Abstract

Background: Ovarian tumors account for 1% of all childhood neoplasms and are the most common genital neoplasm in children. Considering the role of serotonin in the promotion of tumor growth and metastasis in some cancers, tropisetron as a 5-hydroxytryptamine (5-HT3) receptor antagonist can exert anti-neoplastic effects. The current study seeks to determine the association between the use of tropisetron and ovarian cancer-cell lines (SKOV3) by evaluating apoptotic regulators.

Materials and Methods: In this experimental study, after the addition of tropisetron to SKOV3 cancer cells at the concentrations of 1, 10, and 100 µM, the MTT assay was employed to assess the cell viability percentage within 24, 48, and 72 hours. The level of expression of Bcl2 and Bax genes after 24 hours were determined by Real Time-PCR, and Caspase 3 enzyme activity was measured by the ELISA method. Differences between groups were statistically analyzed by one-way analysis of variance (ANOVA). A p-value less than 0.05 is considered to be statistically significant.

Results: Based on the MTT test, tropisetron significantly decreased the viability of SKOV3 cancer cells. It decreased the levels of Bcl2 expression according to real-time PCR results (P =0.0015) but increased the expression levels of Bax (P = 0.011) in SKOV3 cells. Both caspase-3 enzyme activity and the ratio of Bax to Bcl2 expression (Bax/Bcl2) were increased (P = 0.008 and P = 0.011, respectively).

Conclusion: Tropisetron could inhibit tumor cell growth via apoptosis induction and exert proapoptotic effects in an ovarian cancer ¬cell line. Therefore, it seems that it can be considered as a possible chemotherapy drug for ovarian cancer. However, more studies should be conducted in this regard.

Keywords: Apoptosis, Bax, Bcl2, Caspase, Tropisetron

Introduction

Cancer is one of the major social, health and economic problems, which accounts for nearly one in six deaths (16.8%) in general and one in four deaths (22.8%) among non-communicable diseases (NCDs) worldwide in the 21st century (1). Ovarian tumors account for 1% of all childhood neoplasms and are the most common genital neoplasm in children. Germ cell tumors and sex cord stromal tumors are the most prevalent histological type of ovarian cancer in children and adolescents compared to older groups, while epithelial ovarian cancer is the most common form in adult women (2, 3). Furthermore, the majority of patients appear to have a favorable prognosis and to be in the early stages of the disease. The goals of treatment are to restore the uterine and the remaining ovarian function for future fertility and to cure the diseases. The typical course of treatment includes conservative surgery and combination chemotherapy, depending on the tumor stage and the level of aggression (4). Cancer chemotherapy is the administration of cytotoxic chemicals with the aim of eradicating the tumor or at least reducing the tumor burden, thereby reducing tumor-

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related symptoms and possibly extending life. The process of developing new drugs is slow and, at best, only a few drugs are added to the therapeutic arsenal each year (5). Using the existing drugs for new therapeutic purposes can be a promising idea. In this regard, 5-Hydroxytryptamine subtype (5-HT3) receptor antagonists, including tropisetron, are commonly used to control postoperative and chemotherapy-induced vomiting. The beneficial effects of tropistron are gaining increasing importance in the management of a range of diseases, including cancerous tumors, anxiety, multiple sclerosis (MS), stress disorders and neurodegenerative diseases (6, 7). Previous studies have demonstrated the anti-neoplastic effect of serotonin receptor antagonists. This could be related to the serotonin receptors overexpression in various cancer cells (8). Several studies have demonstrated additional possible effects of 5-HT3 inhibitors. *In vitro* and *in vivo* neuroprotective effects of 5-HT3 receptor antagonists have already been documented. The research conducted *in vivo* has demonstrated that 5-HT3 receptor antagonists exhibit remarkable effect on the Central Nervous System (CNS) (9). The immunomodulatory properties of serotonin antagonists are also evidenced by the overexpression of 5-HT receptors on a variety of immune cells such as T lymphocytes and macrophages (8). Increased tumor growth and metastasis in melanoma, prostate, bladder, pancreatic, breast and liver cancers have been linked to serotonin signaling pathways. Furthermore, high levels of serotonin receptor (5-HT1A) have been shown to transform non-tumorigenic cell lines such as NIH3T3 fibroblasts (10). Accordingly, serum serotonin levels can be used as a prognostic marker for prostate, urothelial, and renal cell cancers (11). Stopping the uncontrolled growth of cancer cells is one way to treat the disease. The most successful non-surgical treatment is targeted apoptosis therapy, which takes advantage of the cell death natural mechanism. A prototypical hallmark of cancer is the avoidance of apoptosis (12). The highly regulated process of apoptosis is triggered by both intracellular and extracellular stimuli. The key modulators of intrinsic and extrinsic apoptotic signaling pathways are the caspase cascade and members of the Bcl2 family. The Bcl2 protein family includes two important pro-apoptotic and anti-apoptotic proteins, including Bax and Bcl2 (13). Suppressing anti-apoptotic genes and stimulating pro-apoptotic genes are two common approaches to developing new cancer therapies (14, 15). Death receptor ligands, Bcl2 inhibitors, XIAP inhibition, and Alkylphospholipid (APL) analogs, which function as apoptotic signals, are some of the targets being investigated (16). Any stage in the pathways can be targeted, but there is still no indication of the most effective target (12). Tropisetron, the antiemetic 5-HT3R antagonist, may have broad biological effects, but little is known about its potential anti-tumor activities. This study examined the effects of tropisetron on the ovarian cancer cell line SKOV3 to determine whether this drug can trigger tumor cell death through apoptosis. In addition, the roles of caspase-3, Bax and Bcl2 proteins in tropisetron-induced apoptosis in SKOV3 cancer cells were investigated.

Materials and Methods

Chemicals

Tropisetron, RPMI 640, Dimethyl Sulfoxide (DMSO), and MTT kit were obtained from Sigma Chemical Co. (Sigma–Aldrich GmbH. Sternheim, Germany). Trypsin-EDTA, PBS, and Fetal Bovine Serum (FBS) were purchased from BIO-IDEA (Tehran, Iran). L-Glutamine

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and Penicillin/Streptomycin were obtained from Gibco (A96 K7H7, Dublin, Ireland). The solvents and chemicals were used in analytical grades. Also, cDNA synthesis kits, RNX Plus kits and Mastermix cybergreen were provided by Yekta Tajhiz Azma (Tehran, Iran), Cinnagen (Tehran, Iran), and Parstous (Tehran, Iran), respectively.

Cell culture

The SKOV3 cell line was obtained from the Pasteur Institute of Iran. The protocol of this study was approved by the ethics committee of Shahid Sadoughi University of Medical Sciences (IR.SSU.MEDICINE.REC.1398.129), and all procedures were done in cell culture lab of Pharmacy School of Shahid Sadoughi University of Medical Sciences. In this experimental study, the cells were kept in RPMI 640 supplemented with penicillin/streptomycin (100 U/ml; 100 µg/ml) and 10% FBS. They were then incubated at 37°C in a humidified atmosphere of 5% CO_2-95% air.

Cell survival analysis

Using the 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) method, the cytotoxicity of tropisetron was examined on SKOV-3 cells. Exponentially growing cells were seeded in 96-well plates at a density of 1 × 104 cells/well in an RPMI-1640 medium to prepare them for treatment. To facilitate adherence, tropisetron was then added to the cells after a 24-hour incubation period at 37°C and in 5% CO_2. Various concentrations (1, 10, and 100 µM) of tropisetron were added to the wells, with non-treated cells used as experimental controls. Cell viability was assessed after incubation for 24, 48, and 72 hours. The cells were incubated at 37°C for 4 hours after the addition of 20 µL of MTT solution (5 mg/mL in PBS) to each plate well to perform the MTT assay. Following the incubation period, 150 µL of a DMSO solution was added to each well, and the cells were shaken for ten minutes at room temperature to dissolve the produced formazan. The percentage of the untreated control was used to determine cell viability, and an ELISA plate reader served to measure absorbance at a wavelength of 570 nm. All the experiments were performed twice in triplicate wells (17).

Total RNA extraction and reverse transcription

Using the RNX Plus kit (CinnaGen, Iran), the total RNA was extracted from the tropisetron-treated cells and the untreated control cells according to the manufacturer's instructions. Spectrophotometric techniques were used to measure the concentrations and purity of the RNA (Eppendorf BioPhotometer, Germany). The complementary first-strand DNA (cDNA) was synthesized based on the instructions in the YT4502 kit obtained from Yekta Tajhiz Azma (Tehran, Iran). With the enzyme H MMuLV reverse transcriptase, RNA (1 µg) was used to synthesize cDNA.

Gene expression analysis by real-time polymerase chain reaction

The apoptotic genes expression (Bax and Bcl2) was assessed through a quantitative real-time polymerase chain reaction (qRT-PCR) using a Corbett RG-6000 real-time PCR device. After a master mix was prepared with 5 µl Cyber, 1 µl cDNA, 3 µl H2O and 0.5 µl primer, it was added to a microtube with a strip cap. As an internal control gene, the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene was employed for normalization. The temperature-time cycle was as follows: Step 1: 95°C for 2 minutes, Step 2: 95°C for 10 seconds, Steps 3 and 4: 60°C for 30 seconds. To ensure the specificity of amplification, the reactions were performed for 40 cycles, and the melting curves were analyzed. The forward and reverse primer sequences of 20 bp in length are listed in Table I.

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Measurement of caspase-3 with ELISA kits

The levels of caspase-3 were quantified using an ELISA kit from ZellBio GmbH. After treatment with 10 μM tropisetron for 24 hours, the SKOV3 cells were gently rinsed with cold PBS, separated with trypsin, and then centrifuged at 1000 × g for 5 minutes. The cell debris was removed through the centrifugation of the cells at 2-8°C and 1500 × g for 10 minutes after re-suspending them in fresh lysis buffer at a concentration of 107 cells/ml and washing them with cold PBS for three times. The untreated cells also served as a negative control. Caspase-3 assays were performed in triplicate following the manufacturer's instructions. Each sample, standard, and reagent was handled at room temperature. After the addition of 100 μl to each well, the wells were incubated at room temperature for two hours. To continue, each well was aspirated and cleaned five times. Then, 100 μl of conjugate was added to each well, the solution was kept at room temperature for one hour, and the plates were washed five times. Once 100 μL of the substrate solution was added, the plates were left to rest at normal room temperature for half an hour. Following that, the stop solution was added to each well, and the plates were read at 450 nm and 540 nm for correction.

Data analysis

The comprehensive Rotor Gene Q Series software was used to analyze the real-time PCR data. The raw data were processed through comparative quantification analyses, where the Ct values for each gene were measured in different groups. The 2-ΔΔCT method was also used to analyze the expression levels of the target genes by interpreting the Ct values derived from the real-time PCR results. Subsequently, the gene expression was presented versus the reference or control group. The final results were reported as mean ± standard error of the mean (S.E.M). One-way analysis of variance (ANOVA) served to make the corresponding comparisons. The Tukey posttest was then used to demonstrate the significance of differences between the groups. A P-value below 0.05 was considered statistically significant. All the statistical analyses were performed with GraphPad Prism (GraphPad Software, Inc., Boston, USA).

Results

Inhibitory effects of tropisetron on the growth of SKOV-3 cells

The cytotoxic effect of tropisetron on SKOV3, human ovarian cancer cells, was assessed by the MTT test. The three tropisetron concentrations of 1, 10, and 100 μM were used for 24, 48, and 72 hours. The results showed the dose-dependent inhibition of the growth of ovarian cancer SKOV3 cells after 24, 48, and 72 hours of treatment with tropisetron (Figure 1). As shown in Figure 1, SKOV3 cell growth was significantly inhibited by tropisetron at the concentrations of 10 and 100 μM in comparison to the control group (i.e. untreated cells). It is noteworthy that the effects were more significant at the dose of 100 μM, while the dose of 1 μM had no significant effect.

Real-time qPCR analysis

The effects of tropisetron treatment (10 μM) on the expression levels of Bcl2 and Bax mRNAs were assessed using real-time PCR, and the Bax/Bcl2 ratio was calculated after 24 hours. It was found that treatment with tropisetron could significantly increase Bax mRNA expression in comparison to the control group (P = 0.011) (Figure 2a). Additionally, the expression of Bcl2 mRNA in the tropisetron-treated cells markedly decreased, compared to the untreated cells (P = 0.001) (Figure 2b). As

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a result, the ratio of Bax to Bcl2 in the treated cells increased significantly. After treatment with tropisetron, the ratio of Bax to Bcl2 was observed to be upregulated in the SKOV3 cells (P = 0.011), suggesting that tropisetron may mediate apoptosis in cancer cells via a mitochondria-dependent mechanism.

Effects of tropisetron on caspase-3 levels

The influence of tropisetron on caspase-3 levels was examined to determine whether tropisetron would trigger apoptosis via the caspase pathway. Based on the manufacturer's instructions, caspase-3 levels were measured in triplicate using an ELISA kit. These levels were found significantly increased in the SKOV3 cells after 24 hours of exposure to 10 µM of tropisetron, compared to the control group (P = 0.008) (Figure 3).

Table I: PCR primers for real-time RT-PCR analysis

Primer Name	Sequence (5´–3´)
Bax Forword	**GGG GAC GAA CTG GAC AGT AA**
Bax Reverse	**CAG TTG AAG TTG CCG TCA GA**
Bcl2 Forword	**ATG TGT GTG GAG AGC GTC AA**
Bcl2 Reverse	**ACA GTT CCA CAA AGG CAT CC**
GAPDH Forword	**CAG CCT CAA GAT CAT CAG CA**
GAPDH Reverse	**TGT GGT CAT GAG TCC TTC CA**

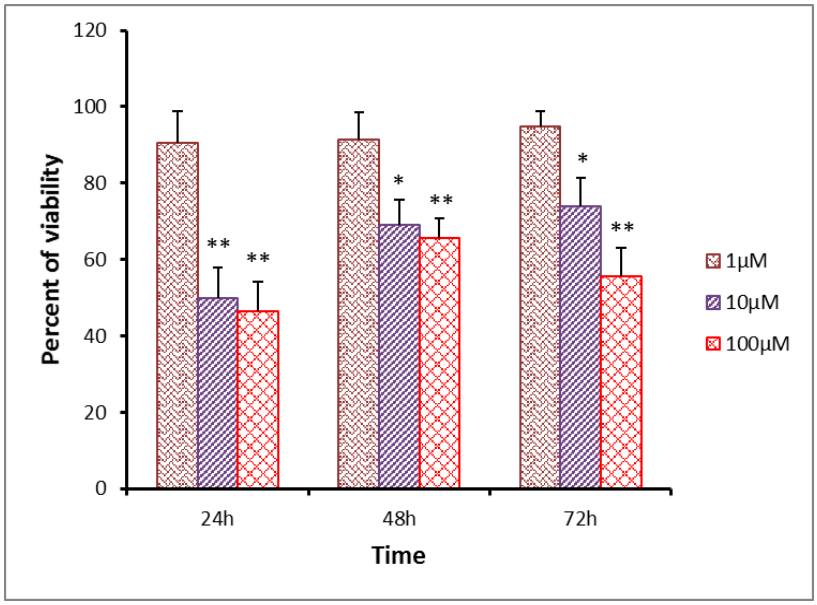

Figure 1. The cytotoxic effects of tropisetron (1, 10,100 µM) in human ovarian cancer cells (SKOV3) after 24, 48, and 72 hours of treatment. Cell viability was assessed using the MTT assay. Each point represents three independent test means ± S.E.M. Statistical significance was considered as *P < 0.05, **P < 0.01.

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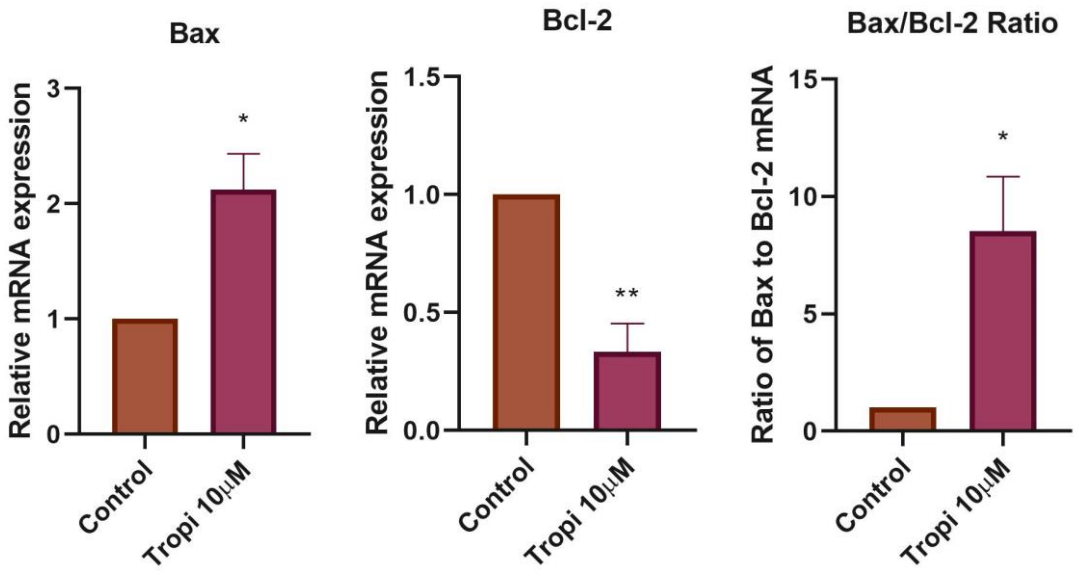

Figure 2. The plasma calprotectin levels are higher in different stages of solid tumors compared to the healthy control group. The highest calprotectin level was observed in the remission phase (209.715±156.13 ng/ml).

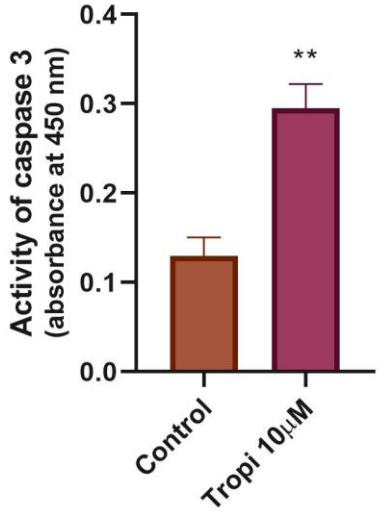

Figure 3: Effects of tropisetron (10 μM) on caspase 3 levels in SKOV3 cell lines after 24 hours of exposure. Data is presented as mean± S.E.M. The caspase 3 level showed a significant difference from the control group (**P < 0.01).

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Discussion

In this study, the effects of tropisetron on ovarian cancer cell lines were examined, and the changes in the expression of Bcl-xL (anti-apoptotic) and Bax (pro-apoptotic) genes as well as the Bax/Bcl-xL ratio and caspase-3 protein levels in SKOV3 cells were reported. Tropisetron is a commonly used medication for vomiting caused by chemotherapy (13). Studies on humans and animals have shown that tropisetron has anti-inflammatory and anti-cancer properties (18). Recently, different publications have reported the anti-cancer effect of tropisetron on experimental models of mouse lung cancer and human breast cancer cell line (MCF-7) (8, 19). Tropisetron showed cytotoxic activity against the SKOV3 cell line in our study, as demonstrated by *in vitro* cell proliferation inhibition in an MTT viability assay. The results of this study support previous research in that tropisetron has anti-tumor and anti-carcinogenic properties through apoptotic pathways that trigger cell death. Numerous studies have shown how neurotransmitters influence cancer cell growth, invasion, dissemination and tumor angiogenesis via their different receptor types (19-21). Serotonin influences immune cells in many ways. It is crucial for the treatment of immunomodulatory disorders and inflammation, including rheumatoid arthritis, allergic asthma, intestinal inflammation and neurodegenerative diseases. Serotonin levels in tumors are critical for tumor growth, according to the genetic models of a range of cancer cells, including lung, melanoma and colon cancers (21). The development of tumor cells in breast cancer is significantly influenced by their connection with neurotransmitters such as serotonin (22). In addition, impairment of the immune system due to the alteration of serotonin receptor (5HTR2A and 5HTR3A) gene expression in peripheral blood mononuclear cells is an important factor in disease progression in breast cancer patients (22). According to Soll et al. (2010), serotonin activates mTOR downstream targets, including p70S6K and 4E-BP1, which, in turn, promotes the growth of hepatocellular cancer tumors. This was determined by assessing the function of serotonin in human hepatocellular cancer cell lines (Huh7 and HepG2) (23). According to Siddiqui et al. (24), serotonin contributes to the growth of tumors in a variety of cancers, such as colon, lung and prostate cancers. It has also been shown to act as a growth promoter in various cancer and non-cancer cell populations. Serotonin blockers, particularly those that target 5HT1A and 5HT1B, not only suppress the spread of cancer cells but also trigger apoptosis in the cancerous cells of bladder and prostate. These inhibitors have been proposed to be used to treat prostate and bladder cancers (25, 26). An investigation by Atayi et al. (2010) demonstrated that the selective 5-HT3 receptor agonist phenylbiguanide had proliferative effects on human colon cancer cell line (HT29 cells), while the selective 5-HT3 receptor antagonist Y25130 hydrochloride had anti-proliferative effects and reduced cell growth. This implies that 5-HT3 receptors may be involved in the pathophysiology of colorectal cancer (27). While 5-HTR4 was mainly detected in high-grade tumors, 5-HTR2B immune responses were strong in both low- and high-grade tumors, prostate intraepithelial neoplasia (PIN), benign prostatic hyperplasia (BPH), and vascular endothelial cells. Dose-dependent inhibition of prostate cancer (PC) cell proliferation was observed by the antagonists of both receptor subtypes. These results suggest that different G protein-coupled receptor subtypes, either present or overexpressed in human prostate cancer cells, mediate the effects of serotonin on PC. This can be of benefit for

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the development of selective inhibitors that target hormone-refractory prostate cancer (HRPC) (28). Hejazi et al. (19) examined the role of serotonin receptors (5-HTR2A and 5-HTR3A) and their antagonists in MCF-7, a human breast cancer cell line. Their study showed the suppression effects of tropisetron (5HTR3A antagonist) and ketanserin (5HTR2A antagonist) on MCF-7 cells proliferation. They found that these agents could lead to the apoptosis of MCF-7, hence a recommended approach for treating breast cancer cells (19). The 5HTR2A and 5HTR3A genes expression in tumoral tissues of women with breast cancer was 3.12 and 3.24 times more than their marginal tissues. Therefore, tumor development, progression and metastasis might be related to the mRNA expression of these receptors (22). According to the above studies, blocking the serotonin receptor inhibits the growth of tumor masses and the angiogenesis of cancer cells (22). A retrospective clinical study showed that the use of 5-HT3 antagonists such as palonosetron and ramosetron in surgical procedures for lung cancer, especially after open thoracotomy, may have anti-cancer properties and improve the recurrence-free survival of patients. This discovery suggests that these drugs should be prioritized as the primary treatment for nausea in people undergoing lung cancer surgery (29). Tropisetron appears to have an anti-tumor effect primarily due to its antioxidant properties. Numerous studies have demonstrated the immunomodulatory and anti-inflammatory properties of tropisetron. In mouse lymphocytes, tropisetron reversed H2O2-induced oxidative stress (8). Tropisetron has been demonstrated to activate the antioxidant kinases JNK and extracellular signal-regulated kinase in hepatocytes from an experimental liver disease in a mouse (30). Tropisetron may also have an antioxidant effect by modulating the iNOS activity (31). Moreover, it activated antioxidant enzymes such as catalase, heme oxygenase-1, and superoxide dismutase (SOD) (32, 33). The drug decreased oxidative stress in the PC12 neuronal cell line when it was exposed to elevated glucose stress by blocking the generation of reactive oxygen species (ROS) (13). A tropisetron-treated lung cancer mouse model showed increased and decreased levels of Interferon gamma (IFN-γ) and Interleukin 4 (IL-4), respectively (8). Furthermore, tropisetron treatment resulted in the significantly decreased expression of β-catenin and cyclooxygenase-2 (Cox-2) as well as the lowered levels of interleukin-1 beta (IL-1β), tumor necrosis factor alpha (TNF-α), toll-like receptor 4 (TLR4) and myeloid differentiation primary response protein 88 (MyD88) in the colitis-associated cancer (CAC) experimental group. These findings imply that tropisetron inhibits inflammation and, thereby, delays the onset of CAC (34). In numerous *in vitro* and *in vivo* studies, Tropisetron has been reported to have anti-apoptotic effects under a variety of conditions. Aminzadeh (2017) found that, by modifying the expression of Bax and Bcl2 proteins in rat pheochromocytoma (PC12) cells, tropisetron increased cell viability and protected the cells from apoptosis caused by hyperglycemia (13). In addition, the 5-HT3 antagonist tropisetron was able to attenuate the development of peripheral neuropathy and cardiomyopathy in diabetes, associated with the attenuation of apoptosis as indicated by Bax and Bcl2 changes (35, 36). A possible mechanism for inducing cell death by therapy in the human breast cancer cell line (MDA-MB-231) is the regulation of Bax and Bcl2 expression with extremely low-frequency electromagnetic fields (ELF-EMFs). This approach raised the levels of Bax protein while decreasing the expression of Bcl2

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(37), which was consistent with the *in vitro* findings in this study. The proteins belonging to the Bcl2 family have the ability to either stimulate or inhibit apoptosis. According to some studies, the susceptibility to apoptosis in cells is determined by the ratio of pro- to anti-apoptotic proteins (38). The same studies also examined the influence of tropisetron on the apoptosis pathway. In one study, the melanoma cell lines WM-266–4 and B16F10 demonstrated selective concentration-dependent cytotoxicity when exposed to ondansetron and tropisetron by impairing microtubule formation. The drugs did not cause appreciable levels of transient cell cycle arrest but increased the classic apoptotic features of phosphatidylserine exposure, cleaved caspase-3, subG1 DNA accumulation, and mitochondrial membrane permeability. Furthermore, the cytosolic calcium level of melanoma cells was increased, phosphorylated ERK1/2 was triggered, and NF-κB was inhibited. Based on docking studies, it was expected that these agents would bind to the colchicine binding site of the tubulin molecule. When administered in conjunction with chemotherapy, antiemetic drugs can enhance the cytotoxic effects of the treatment after they are successfully delivered to the tumor site (39). Tropisetron reduced cell damage in high glucose (HG)-induced human retinal pigment epithelial cell line (ARPE-19) cells, increased cell viability, and reduced lactate dehydrogenase (LDH) release. Tropisetron treatment partially inhibited the excessive production of TNF-α, IL-1β, interleukin-6 (IL-6), ROS, and malondialdehyde and reduced the superoxide dismutase activity caused by HG. The treatment also prevented HG-induced cell apoptosis, which was accompanied by the upregulation of proapoptotic proteins and the downregulation of anti-apoptotic proteins. In ARPE-19 cells, HG caused the loss of SIRT1 and an increase in ROCK1; this reversed after tropisetron treatment (40). By controlling the SIRT1/ROCK1 axis, tropisetron protected against HG-induced inflammation, oxidative stress, and cell death in ARPE-19 cells. This suggests the therapeutic potential of tropisetron to reduce retinal pigment epithelial cell damage in diabetic retinopathy; the results of the present study support this theory. The study has assessed the effect of tropisetron on apoptotic regulators. The findings suggest that tropisetron may be effective in modulating the expression levels of Bax and Bcl2 genes as well as caspase-3 levels in a SKOV3 cell line. As the MTT assay results indicated, tropisetron induced cytotoxic effects in the SKOV3 cell line over 24, 48 and 72-hour periods in dose-related patterns. In agreement with the result of this study, the phosphorylation, gene expression, and apoptosis of the Bcl2 protein in docetaxel (DTX)-loaded human serum albumin (HSA) nanoparticles (NPs) against breast cancer cell lines were examined. Following medication, it was discovered that breast cancer cells were stopped in the G2/S phase and that Bcl2 phosphorylation was suppressed (38). Furthermore, it appears that many of the molecular and structural changes in apoptosis are caused directly by the activation of caspases. Mousavi et al. examined the Bax gene and caspase proteins in MCF-7 cells following saffron extract treatment. They found that saffron extract triggered apoptosis of cancer cells and increased the expression of the protein Bax in those cells; therefore, it can be used in breast cancer chemotherapy (41, 42). In agreement with earlier studies, it was found in this study that tropisetron significantly increased the pro-apoptotic Bax, the ratio of Bax to Bcl2, and caspase-3 in SKOV3 cells. There was also a decline in anti-apoptotic Bcl2, suggesting a possible involvement of

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the Bcl2 family in the anti-cancer properties of tropisetron. Further research is needed to prove the role of 5-hydroxytryptamine and particularly 5-hydroxytryptamine3 receptor antagonists as potential anti-neoplastic agents. This study faced limitations mainly due to the lack of funds to measure the expression of Bax and Bcl2 proteins.

Conclusion

In summary, the present investigation demonstrates the toxic effect of tropisetron on the SKOV3 cell line as well as its association with programmed cell death or apoptosis. Therefore, serotonin-related signaling pathways may offer a potential therapeutic agent for the treatment of ovarian cancer. To completely understand the mechanisms underlying cell death, more investigation is needed.

Ethical Considerations

The protocol of this study was approved by the ethics committee of Shahid Sadoughi University of Medical Sciences (IR.SSU.MEDICINE.REC.1398.129), and all procedures were done in cell culture lab of Pharmacy School of Shahid Sadoughi University of Medical Sciences.

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Authors' Contributions

FT and SJ made conception and design of the work; FT, FA, and SJ contribute to conception of the work, participated in the acquisition, analysis, and interpretation of data, FT and SJ wrote the main manuscript text and supervised data interpretation; All authors reviewed, approved, and accept responsibility for this manuscript.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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