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Research Article

Investigation of the Effects of Cetuximab and Agomelatine Drugs on Proliferation and Apoptosis in Prostate Cancer Cells

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Abstract

Cancer is a complex health problem that occurs as a result of uncontrolled division of cells and includes a series of diseases that depend on evidence and environmental factors. There are more than 100 types of cancer known worldwide. This study used prostate cancer cells, which are the most common disease in men and cause 1,600,000 cases and 366,000 deaths annually. During the cancer treatment process, different medications are used in most cancer patients. In this context, this study examining the proliferation and apoptosis effects of the chemotherapeutic agent Cetuximab (CTX) and the antidepressant Agomelatine drugs on the prostate cancer PC3 cell line emphasizes the great importance of psychological motivation in the treatment process. Focusing on the characteristics and prevalence of prostate cancer, the effects of Cetuximab and Agomelatine on prostate cancer cells were examined.

This research was conducted to understand how the drugs Cetuximab and Agomelatine affect the proliferation and apoptosis in prostate cancer cells. The findings suggest that these drugs may provide potential effects in the treatment of prostate cancer.

Keywords: Prostate cancer, cetuximab, agomelatine, PI3K/AKT/mTOR pathway, gene expression

Cetuximab ve Agomelatin İlaçlarının Prostat Kanseri Hücrelerindeki Proliferasyon ve Apoptoza Etkisinin İncelenmesi

Öz

Kanser, hücrelerin kontrolsüz bölünmesi sonucu ortaya çıkan, delillere ve çevresel faktörlere bağlı bir dizi hastalığı kapsayan karmaşık bir sağlık sorunudur. Dünya çapında bilinen 100'den fazla kanser türü vardır. Bu çalışmada, yılda 1.600.000 vaka ve 366.000 ölümle sonuçlanan ve erkeklerde en sık görülen prostat kanseri hücreleri kullanılmıştır. Kanser tedavisi sürecinde çoğu kanser hastasında farklı ilaçlar kullanılmaktadır. Bu bağlamda kemoterapötik ajan Cetuximab (CTX) ve antidepresan ajan Agomelatin ilaçları ile prostat kanseri PC3 hücre hattı üzerindeki proliferasyon ve apoptoz üzerindeki etki ile tedavinin önemine odaklandı. Prostat kanserinin özelliklerine ve yaygınlığına odaklanılarak Cetuximab ve Agomelatin'in prostat kanseri hücreleri üzerindeki etkileri incelendi.

Bu araştırma, Cetuximab ve Agomelatin ilaçlarının prostat kanseri hücrelerinde proliferasyonu ve apoptozu nasıl etkilediğini anlamak amacıyla yapıldı. Bulgular, bu ilaçların prostat kanseri tedavisinde potansiyel etkiler sağlayabileceğini düşündürmektedir.

Anahtar Kelimeler: Prostat kanseri, setuximab, agomelatin, PI3K/AKT/mTOR yolağı, gen ekspresyonu

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1. Introduction

Prostate cancer (PC) is one of the most common types of cancer in men worldwide and causes significant health problems (1). Prostate cancer occurs due to the proliferation and growth of cells in the prostate gland. This type of cancer is associated with various pathophysiological, environmental factors and genetic factors mechanisms (2).

It is the tight expression status of the Epidermal Growth Factor Receptor (EGFR), which is one of the most important factors in the pathogenesis of prostate cancer (3). EGFR is intricately involved in processes such as apoptosis, differentiation process and cellular proliferation (4). Accordingly, monoclonal antibodies such as the therapeutic agent Cetuximab, which targets EGFR, are emerging as a different and important strategy for the treatment of this type of cancer (5, 6).

However, drugs such as Cetuximab, which has a strategic importance, may cause resistance to these and similar treatments over time, despite the initial positive responses for prostate cancer (7, 8). Accordingly, additional treatment or alternative strategies are needed and this emerges as an important issue for the treatment of prostate cancer.

Melatonin regulates mitochondrial functions in cancer cells, reduces reactive oxygen species (ROS) levels, and suppresses tumor growth by arresting the cell cycle via p53/p21 pathways (9, 10, 11). In prostate cancer cells, melatonin activates mitochondrial pathways and triggers apoptosis. It increases the Bax/Bcl-2 ratio, leading to disruption of mitochondrial membrane potential and cytochrome C release. This process induces cell death by activating caspase-3 and caspase-9 (12).

The effects of melatonin on cellular metabolism, cell cycle, metastasis, oxidative stress, apoptosis, and circadian rhythm have been investigated in detail in the context of prostate cancer (13). It has been reported that the disruption of circadian rhythm is associated with the progression of prostate cancer, and melatonin has been shown to suppress the growth and spread of cancer cells by regulating this rhythm. However, more in vivo and in vitro studies are needed to better understand the mechanisms of these effects (12,13).

Although the effects of melatonin on prostate cancer have been widely studied in the literature, there is no direct study on agomelatine. Agomelatine is an analog of melatonin and has been studied in various types of cancer. This drug, used in the treatment of depressive disorders, may affect important mechanisms in cancer pathogenesis by regulating the circadian rhythm (14). However, more research is needed to elucidate the effects of agomelatine on prostate cancer. The literature also emphasizes that melatonin may affect hormonal regulation and cellular differentiation in prostate cancer cells (12).

This study aims to understand the proliferation and apoptosis effects of drugs on PC3 prostate cancer cells. It is aimed to examine the cellular and molecular responses in detail and explain the pathways. These contribute to knowledge in prostate cancer treatment and research and provide an environment for the development of sustainable treatment strategies.

2. Material and Methods

2.1. Cell Culture

The prostate cancer PC3 cell line used in this study is stored in at -196 °C in Erzurum Technical University, High Technology Application and Research Center (YUTAM), Molecular Cancer Biology Laboratory.

For the PC3 cell line medium, 10% fetal bovine serum (FBS), 1% ml of penicillin-streptomycin (Pen-Strep), 2% L-Glutamine was added to RPMI. The prepared media were stored in 50 ml falcon tubes under +4 °C conditions. Parenteral PC3 monolayer cell lines were incubated in a cell culture medium at 37°C with 5% carbon dioxide (CO_2) and 95% humidity in an incubator (Esco Co., Korea) in 25 cm² flasks under sterile conditions to facilitate cell proliferation.

The cell line, which was kept frozen, was kept in a 37 °C water bath until it was thawed. Then, 3-4 ml of the prepared medium, previously kept at 37 °C, was added into a 15 ml centrifuge tube and centrifugation was performed at 1300 rpm for 5 minutes. Afterwards, the supernatant was removed and the pellet remaining in the centrifuge tube was resuspended in the medium and transferred to a total of 4 ml of medium in a 25 cm^2 flask.

2.1.3. Passaging of Cells (Subculturing)

Cells that had proliferated and reached a certain confluency (80%) in the cell line were first washed with 2 ml of PBS buffer and then 3 ml trypsin/EDTA (Ethylene Diamine Tetraacetic Acid) was added and kept in a CO_2 incubator for 7-10 minutes to separate the cells from the surface. Then, 8 ml of medium was added and centrifuged at 1300 rpm. The supernatant was removed from the centrifuged cell suspension. 10 ml of medium was added to the pellet and transferred into a 25 cm^2 flask. The cells whose passaging process was completed were incubated again at 37 °C in a 5% CO_2 incubator with 95% humidity.

2.2. Drug Treatment

2.2.1. Cetuximab and Agomelatine Treatments

Prostate cancer cells (PC3) are prepared together with Agomelatine (Valdoxan, Thymanax, AG0178) and Cetuximab (IMC-C225, Erbitux) cell medium with a concentration of 0.3 µg/ml, 3 µg/ml, 50 µg/ml and cetuximab 50 µg/ml. The concentration used in combined and single study groups are listed in Table 1.

2.3. MTT Assay for Cell Viability

For MTT analysis, 1500 cells were seeded into each well into 2 different plates with 96 wells. Afterwards, it was left for incubation at 37 °C in an oven with 5% CO_2 for 24 hours and 48 hours. MTT (cytotoxicity) analysis was performed to determine the cell viability. The kit was used for this analysis (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, Sigma Co. USA) (6-7).

2.3.1. Assay Procedure

MTT analysis is a common colorimetric cytotoxicity test that is frequently used to determine cell viability and results are measured using a spectrophotometer (15). The effects of any therapeutic agent to be used in the MTT method on the desired cell can be determined as cytotoxic or proliferative (16). The MTT kit is used according to its instructions and the possible cytotoxic effect on the PC3 cell line is measured. This method is based on the principle of colorometric evaluation of the color change in cells incubated with the MTT agent (17).

Agomelatine and/or cetuximab drugs, prepared as serial dilutions kept in the incubator for 24 hours and 48 hours, were divided into groups in series and added to the cell medium. The first column of the A series was set as the cell control and the second column of the A series was set as the medium control. MTT method was then applied to the cells treated with agomelatine and cetuximab, which were incubated for 24 and 48 hours.

Cells were seeded at a density optimized for the detection of cellular metabolic activity and subsequently treated with the drugs. Post-treatment, MTT solution was added, and cells were incubated to facilitate the formation of formazan crystals. The crystals were dissolved, and the absorbance was measured at 570 nm spectrophotometrically. Data were normalized and analyzed to determine the effects of treatments on cellular viability (Multiscan, Thermo, USA).

Based on the results of this analysis, the doses of agomelatine and dose of cetuximab were determined according to previous studies (18, 19).

When these drugs were used together, two combined doses were administered. Afterwards, drug applications at doses appropriate to the determined values were applied to the passaged flasks and incubated for 24 and 48 hours, and these cell flasks were stored for use in qRT-PCR analyses.

2.4. Quantitative Real-Time PCR (qRT-PCR) Analysis

2.4.1. RNA Isolation

Total RNA from PC3 prostate cancer cells treated with drugs was isolated using a commercially available kit according to the manufacturer's instructions (Ambion RNA Mini Kit, USA). The RNA isolation procedure followed a series of necessary steps including cell lysis, homogenization and purification, and a detailed protocol was implemented to ensure the integrity and purity of the isolated RNA. The concentration and purity of the obtained RNA were determined spectrophotometrically using the Nanodrop device (EPOCH Take3 Plate, Biotek), and the RNA samples were stored at -20 °C.

2.4.2. cDNA Synthesis

After RNA isolation, cDNA synthesis was performed using the Maxime RT Premix Kit. The synthesis protocol involves mixing 5 μL of RNA and 15 μL of RNase-free water. Reactions were performed using a Veriti 96-well thermal cycler (Applied Biosystem) with temperature

settings of 45 °C for 60 min and 95 °C for 5 min. Following synthesis, cDNA samples were measured spectrophotometrically and stored at −20 °C.

2.4.3. qRT-PCR Analysis

qRT-PCR analysis Gene expression analysis of EGFR, Bcl-2, Bax, AKT3, PIK3CA, MTOR and GAPDH genes was performed by qRT-PCR using primers specifically designed for each gene (see Table 1 for primer sequences).

Amplification, detection, and data analysis were performed using the Qiagen Rotor-Gene real-time PCR system (Rotor-Gene Q 5plex HRM system) to ensure specificity, efficiency, and reproducibility of the results. Amplification conditions were set at 95°C for 3 min for enzyme activation, 95°C for 5 s (40 cycles) for denaturation, and 60°C for 10 s (40 cycles) for amplification. Relative gene expression was calculated using the ΔΔCT method and provided insight into the molecular mechanisms of cellular responses to drug treatment.

Table 1. List of RT-qPCR primers used for RT-PCR analysis

PRIMER	5'-3' PRIMER SEQUENCES	
BAX	Forward	CGCATCCTGAGGCACCGG
	Reverse	TTTCATCCAGGATCGAGCAGGG
GAPDH	Forward	GGTCAATGAAGGGGTCATTGATGG
	Reverse	ATTTGGTCGTATTGGCGCCT
EGFR	Forward	TCGTTGGACAGCCTTCAAGACC
	Reverse	AACACCCTGGTCTGGAAGTACG
BCL	Forward	CGCATCCTGAGGCACCG
	Reverse	TTTCATCCAGGATCGAGCAGGG
AKT3	Forward	GGAAGAATGGACAGAAGCTATTCCA
	Reverse	TCCACTTGCCTTTCTCTCGAAC
MTOR	Forward	GTCAGTGGGACAGCATGGAAG
	Reverse	CCCATATGCCCGACTGTAACTC

Statistical analyses

MTT and RT-PCR data are expressed as mean ± standard deviation. Normality analysis was performed using the Shapiro-Wilk test (SPSS 20.0). Differences between groups were determined using the Kruskal-Wallis test. A p value of <0.05 was considered statistically significant.

3. Results and Discussion

3.1. Invert microscopic analysis

MTT analysis provided detailed information about the cell morphology and population density of post-treatment PC3 prostate cancer cells viewed with an inverted microscope. Detailed analysis revealed that survival was significantly reduced in the Agomelatine and Cetuximab treatment groups, with particularly pronounced effects observed at certain doses.

Additionally, the results revealed the possibility of dose- and time-dependent cytotoxic effects of the administered drug. The pooled results of the MTT analysis provide important information to further investigate the potential of Agomelatine and Cetuximab when used alone or in combination as therapeutic agents in the treatment of prostate cancer. The morphology of PC3 cells for the 24 and 48-hour incubation period is seen in Figure 1.

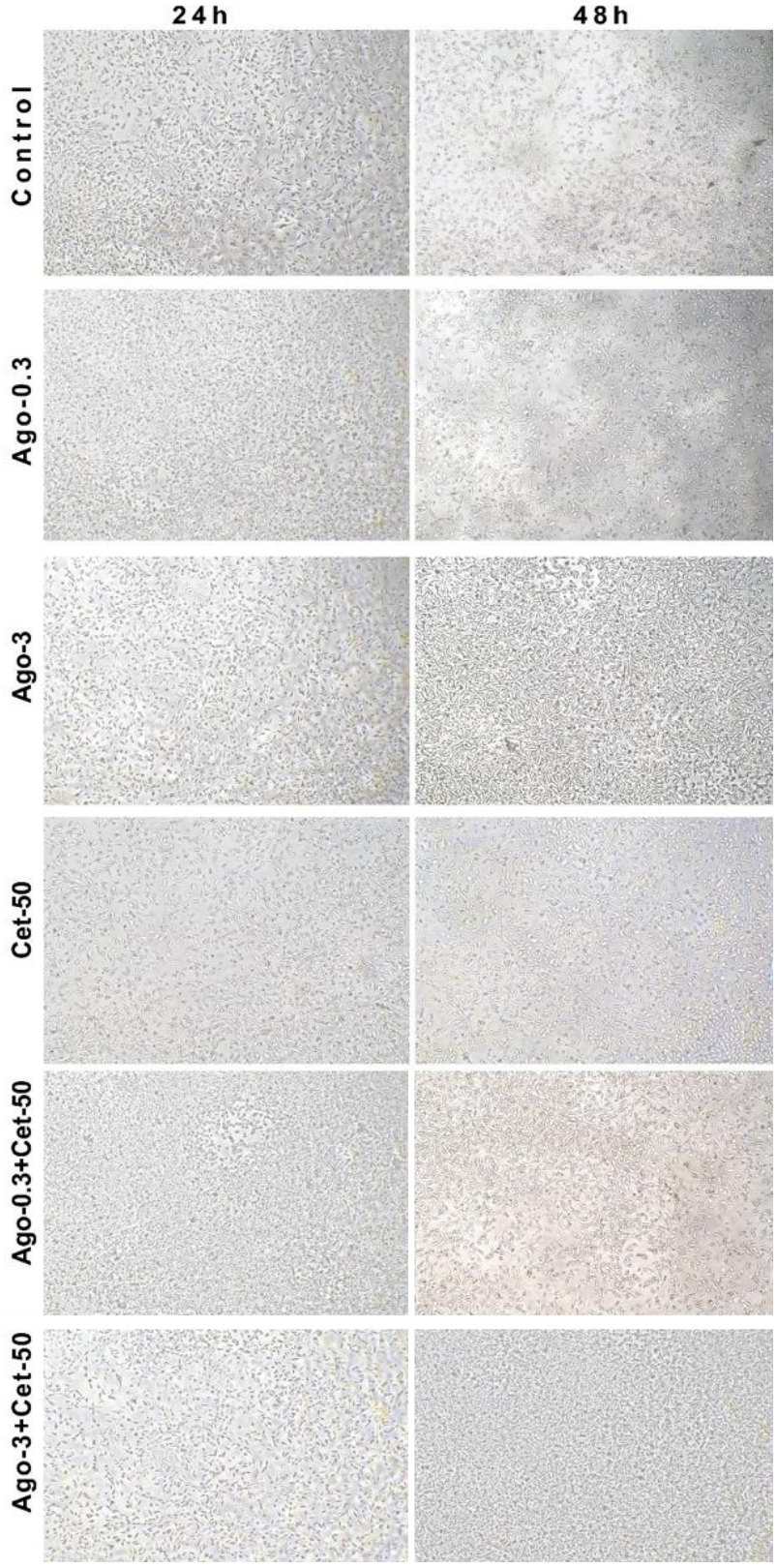

Figure 1. PC3 cells belonging to groups in Agomelatine and/or Cetuximab drug application on an inverted microscope for 24 and 48 hour MTT analysis.

3.2. Cytotoxicity Analysis

The cytotoxic effects of Agomelatine and Cetuximab, alone and in combination, on the PC3 prostate cancer cell line were evaluated by comprehensive analysis. Figure 2 presents the cytotoxicity results obtained when PC3 cells were treated with various dosages of Agomelatine and Cetuximab and then incubated for 24 and 48 hours.

The results show that cell viability was significantly reduced in all treatment groups compared to the control group and varied in a highly dose- and time-dependent manner. In particular, administration of agomelatine at doses of 0.3 μg/ml (Ago-0.3) and 3 μg/ml (Ago-3) significantly reduced cell viability after 24 and 48 h of incubation. Similarly, cetuximab (Cet-50) at a dose of 50 mcg/ml showed strong cytotoxic effects, especially when used in combination with both doses above. Co-administration of Agomelatine and Cetuximab showed significant synergistic effects, especially during the 48-hour incubation period. Cytotoxicity was not only additive but also showed an enhanced effect; this suggested an interaction between the apoptotic and proliferative pathways affected by the two drugs.

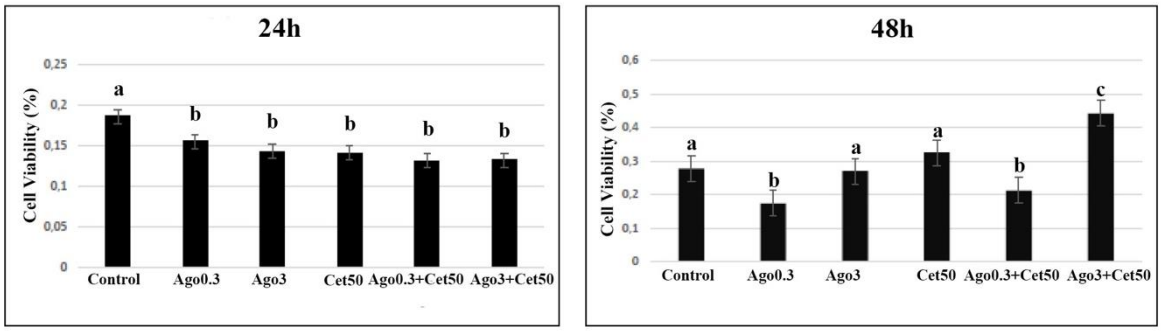

Figure 2. Cytotoxicity results of PC3 prostate cancer cell lines treated with different dosages of agomelatine and/or cetuximab and then incubated for 24 and 48 hours. The data were expressed as mean ± standard deviation. The letters (a,b,c) indicate the differences between the groups. P value of <0.05 was considered statistically significant.

3.3. Real Time-PCR Analysis Results

Agomelatine and/or Cetuximab interact with EGFR protein at different concentrations and durations, enabling communication between many cancer cells. EGFR, which regulates cell growth and division, is associated with abnormal expression of this gene in multiple types of cancer, such as prostate cancer. When we examine Figure 3, it explains and allows us to observe the EGFR gene expression values for PC3 prostate cancer cells subjected to different concentrations of Agomelatine and Cetuximab drug treatments and durations. When the 24-hour incubated results in the first period were examined, when the Control group was evaluated with the GAPDH gene, the highest EGFR/GAPDH ratio was observed in this period group.

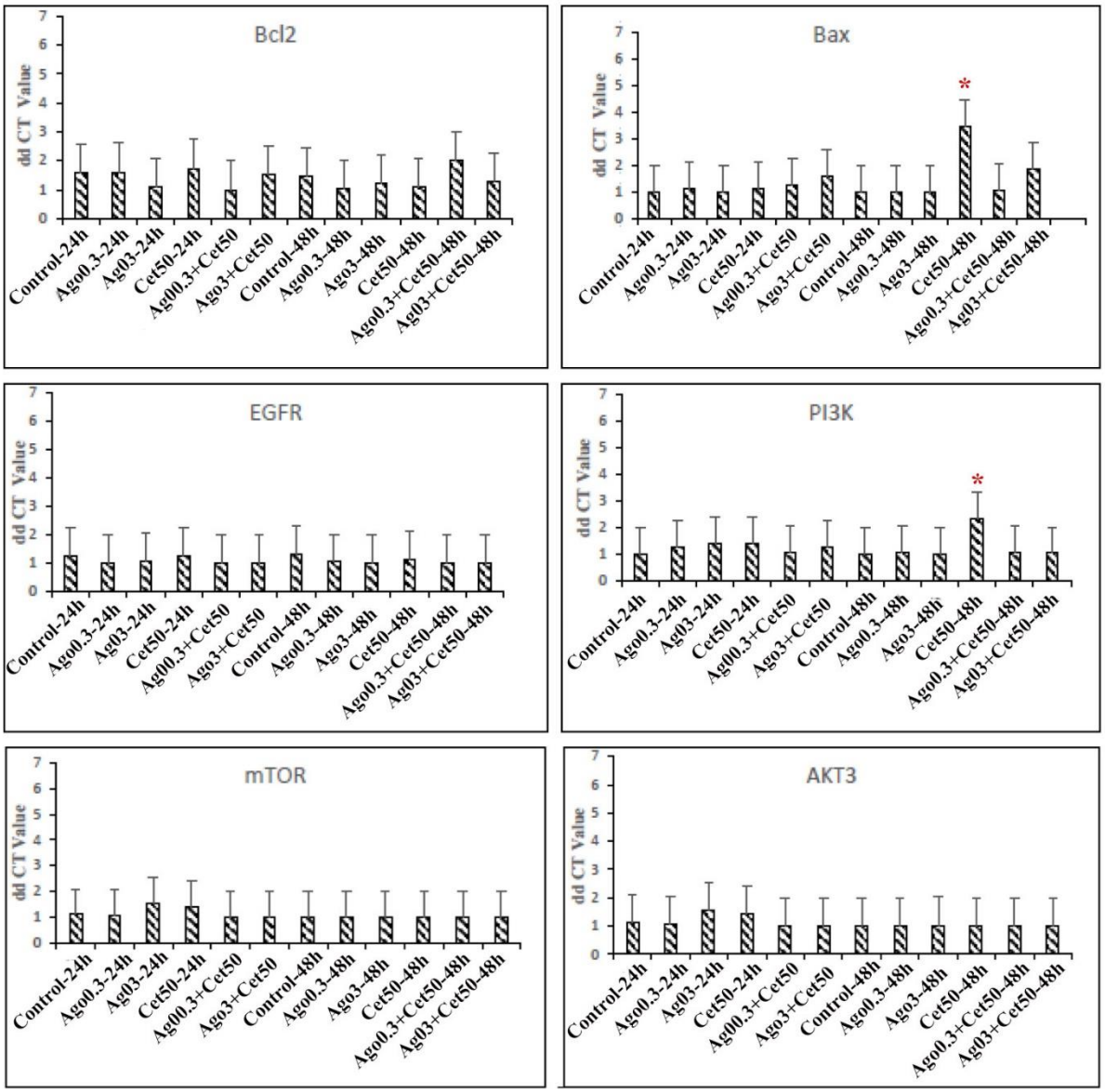

Figure 3. Gene Expression Values of EGFR, PIK3CA, BCL, BAX, mTOR, and AKT3 in PC3 Prostate Cancer Cell Lines Incubated for 24 and 48 Hours with Various Doses of Agomelatine and Cetuximab. The data were expressed as mean ± standard deviation. The asterisk indicates the differences between the groups. P value of <0.05 was considered statistically significant.

EGFR Gene Expressions

In the study, it was observed that the level of EGFR mRNA expression decreased in the groups administered Agomelatine and/or Cetuximab compared to the control groups. The mRNA expression levels are seen in Fig 3.

PIK3CA Gene Expression

It was observed that PI3K mRNA expression increased more in the Ago3 and Cet 50 groups with 24 hours incubation. It was determined that the increase in the distribution of Agomelatine and Cetuximab in the other 24-hour incubation groups was limited compared to the control

group. PI3K mRNA expression level was determined to be highest in the Cet-50 group with 48 hours incubation (Fig 3).

BCL-2 Gene Expression

While it was determined that Bcl-2 mRNA expression increased in the Ago0.3, Cet-50 and Ago3+Cet 50 groups compared to the control group, mRNA expression in the other groups was determined to be close to the control. Bcl-2 mRNA expression was observed to be at the highest level in the Ago 0.3 +Cet-50 group (Fig 3).

BAX Gene Expressions

It was observed that the mRNA expression level of the BAX gene increased with Ago3 application with an incubation period of 48 hours compared to the control group. It was observed that mRNA levels were close to the control in other groups (Fig 3).

mTOR Gene Expressions

An increase in mTOR mRNA levels was observed in the 24-hour Ago3 and Cet50 groups compared to the control. No significant change was detected in the mTOR mRNA expression levels measured in the other groups and as a result of 48-hour applications compared to the control groups (Fig 3).

AKT3 Gene Expressions

An increase was observed in the Ago3 and Cet50 groups with 24 hours of incubation compared to the control. No change was detected in Akt3 mRNA levels in all other groups (Fig 3).

4. Discussion

Prostate cancer is one of the most common types of cancer in men worldwide and continues to be an important health problem. This type of cancer usually occurs as a disease without visible symptoms and early diagnosis is usually not easy. As cancer progresses, symptoms such as problems in the urinary tract, difficulty urinating, or frequent urination occur (20). For the diagnosis of this type of cancer, regular health checks are required for men in certain age groups. Epidermal Growth Factor (EGF) is a group of proteins that regulate processes such as growth and division in cancer cells (4). Cetuximab, a biological drug used in cancer treatments (21), aims to prevent the growth and proliferation of cancer cells by bonding with the receptor on the cell surface called epidermal growth factor receptor (EGFR). Recently, antidepressant agents can be used as an additional treatment for problems related to mood disorders encountered in cancer patients. Agomelatine, a drug recently used in the treatment of major depressive disorder, has a mechanism that affects serotonergic and melatonergic receptors (22, 23).

Generally, in people diagnosed with cancer, chemotherapy drugs are used for treatment, and in addition to these drugs, drugs such as antidepressants are used to prevent antidepressive disorders (24, 25). Our study aimed to enrich the literature by evaluating the yet undiscovered

treatment effectiveness of Cetuximab, a chemotherapeutic agent, and Agomelatine, an antidepressant additional treatment method, on prostate cancer cells at different concentrations and different incubation periods. This study investigates the effects of single or combined use of 3 μg/ml and 0.3 μg/ml Agomelatine and 50 μg/ml Cetuximab on apoptotic cell death and cell cytotoxicity for the in vitro PC3 prostate cancer cell line under 24- and 48-hours incubation. Cell cytotoxicity was determined using the MTT method, which is a colorimetric test. Duplicate groups with EGFR, BAX, BCL2, PIK3CA, mTOR and AKT3 genes were analyzed by real time PCR.

In the study, 6 separate groups were created to better understand the individual and combined effects of cetuximab and/or agomelatine drugs on the cell line. These groups were designed as 1. Control group, 2. Ago0,3 group, 3. Ago3 group, 4. Cet50 group, 5. Ago0,3 + Cet50 group, 6. Ago3 + Cet50 group. As a result of the applications, the division and proliferation of prostate cancer cells have been significantly reduced. In addition, according to the 24-h incubation data of the drugs, it was determined that there was a big difference in combination drug use. According to the 48h cytotoxicity data, it is observed that cell growth is significantly reduced in the low dose of agomelatine drug and in the combination of this group with cetuximab. After determining the antiproliferative role of drugs on prostate cancer cells, protein and mRNA isolations were performed using the same drug doses.

PCR was performed as the second step to investigate the proliferation and apoptosis effects of cetuximab and/or agomelatine drugs at different doses and concentrations applied to PC3 prostate cancer cells. Expression values were determined by subjecting the effects of 6 different primers to multiple tests. As a result of these data, the expression value of these drugs on the bcl-2 gene is high in the 24h Ago0,3, Ago3 and Cet50 groups and the 48h Ago0,3, Ago3, Cet50, Ago3 + Cet50 groups, showing that we have achieved the expected positive result. The drugs we use, cetuximab and/or agomelatine, increased the effect of the Bcl-2 gene. Ago3 + Cet50, one of the 24-h incubated groups, also has high expression values on the Bax gene of prostate cancer PC3 drug-treated cells. The group with the highest expression value in the groups as a result of 48h incubation is Cet50. The group with the lowest expression value in 24h and 48h incubations is Ago3. This is generally a positive and desired outcome for these values. It was observed that the expression values of the EGFR gene of PC3 prostate drug-treated cells were high in the Ago0,3, Ago3 and Ago0,3 + Cet50 groups as a result of 24h incubation. It was determined that expression values were high in all groups of 48h incubated cells (Ago0,3, Ago3, Cet50, Ago0,3 + Cet50, Ago3 + Cet50). There is no increased expression of the PI3K gene in PC3 prostate cancer drug-treated cells in the 24h and 48h groups. The expression of the 48h Cet50 group increased in the opposite direction. According to the 24-h incubation data of the groups formed with cetuximab and agomelatine drugs in different doses and combinations, it was observed that the expression value of the mTOR gene increased in the Ago3 and Cet50 groups. ddCT values of 48h incubation cells are close to each other and the expression value is not positive. It is observed that the expression values of the last primer, AKT3 gene, in drug-treated prostate cancer cells are high in Ago0,3 and Ago0,3 + Cet50 of the 24h incubation groups. It is shown that the values of the AKT3 gene in the groups incubated for 48 h are close to each other and ineffective.

The apoptotic effects induced by Agomelatine in our study are consistent with similar results observed in prostate cancer cell lines in the literature (26). Melatonin has antitumoral effects in many types of cancer by contributing to the maintenance of metabolic homeostasis through the regulation of circadian rhythm. These effects are associated with reducing cellular oxidative stress, improving mitochondrial functions, and activating apoptotic pathways (17, 23). In addition, melatonin is reported to arrest the cell cycle and suppress tumor growth via the p53/p21 pathways (22). These findings demonstrate that combinations of cetuximab and agomelatine is more effective on apoptotic mechanisms in prostate cancer cell lines and indicate that these molecular mechanisms should be supported by more comprehensive studies.

As a result of these data, it was determined that although the Bcl-2 gene from 6 different primers applied to different doses and combinations of cetuximab and/or agomelatine drugs in PC3 prostate cancer cells was antiapoptotic, the applied drugs had an effect and led the cancerous cells to apoptosis. This shows the desired positive effect of the cetuximab and/or agomelatine drugs used on cancer cells. In the results drawn for the Bax gene, the desired positive result of cetuximab and/or agomelatine drugs could not be achieved due to the low values in the general groups. Since the EGFR gene is a proliferation gene, it is observed that most of the drug groups that are expected to be suppressed show pressure on EGFR. Therefore, it can be thought that the drugs and doses used may slow down cell growth and proliferation. Since PI3K, mTOR, AKT3 genes are genes that trigger proliferation, they are indicated in the graph with the values at which they could not initiate proliferation in all groups administered with cetuximab and/or agomelatine drugs in different doses and combinations. The data on these genes show that cetuximab and/or agomelatine drugs may provide positive results in slowing or stopping the division of PC3 prostate cancer cells and driving the cancerous cells into apoptosis.

5. Conclusion

This study was conducted to determine the temperature of different methods of cetuximab and/or agomelatine drugs on PC3 cell proliferation. The results of the study revealed that there was a significant decrease in the viability of human PC3 prostate cancer cells after separate and combined applications in some groups. However, when the general effect of these applications on apoptosis was investigated, it was seen that apoptosis was stimulated. The suppression of proliferation and induction of apoptosis observed in some experimental setups provides new horizons in the knowledge of the mechanistic basis of survival and death of PC3 cells in response to this treatment.

Based on the results, it seems that new treatment screening processes can be carried out by continuing with genes and mechanisms related to apoptosis and survival. In order to obtain a definitive and complete result, more detailed studies are needed on this subject, both in vitro and in vivo. Apart from these, the current study reveals that cetuximab and/or agomelatine drugs and their services may present a promising additional treatment opportunity in addition to prostate cancer drug treatments.

Ethics in Publishing

There are no ethical issues regarding the publication of this study

Author Contributions

KA, HTK, RK, BNG and AK contributed to project conception, laboratory works and study design. Writting the article (KA, HTK, AK). All authors read and approved the manuscript. KA; Kevser ALBAYRAK, HTK; Halime TOPAL KIZILOĞLU, RK; Rukiye KÖSE, BNG; Betül Nur GÜNEY, AK; Adem KARA.

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