

The Effect of Radiotherapy on Ether À-Go-Go Potassium Channel (Kv1.10) Conductivity in DU145 Prostate Cancer Cells

Radyoterapinin DU145 Prostat Kanseri Hücrelerinde Ether À-Go-Go Potasyum Kanal (Kv1.10) İletkenliği Üzerine Etkisi

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ABSTRACT

Objective: Prostate cancer is the most common male urogenital system cancer in Turkey. After the lung cancer, it is the second most common cancer among all cancer types. Radiotherapy is one of the methods used in the treatment of prostate cancer. However, prostate cancer cells create resistance to radiotherapy for reasons that have not yet been fully explained. In this study, it is aimed to investigate the effect of radiotherapy on EAG1 potassium channel conductivity.

Methods: DU145 prostate cancer cell line was used in the study. Cells were divided into two groups as control and radiotherapy (RT). Cells in the RT group exposed to a single dose of 6 Gy RT. However, no treatment is taken on the cells in the control group. 120 minutes after application, EAG1 channel currents were recorded using the "whole cell patch-clamp technique" for both groups. Then, the current-voltage curves were drawn for each record and the curves were fit to a line equation. Channel conductivity was obtained by calculating the slope of the line.

Results: In terms of channel conductivity, statistically no significant difference was found between the control group and the RT group.

Conclusion: In conclusion, it has been observed that RT at 6 Gy dose has no effect on EAG1 channel currents, which are expressed at high levels in cancer cells and play an important role in cancer cell proliferation and migration.

Keywords: Prostate cancer, radiotherapy, patch-clamp, oncochannels, EAG1 channels.

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ÖZET

Amaç: Prostat kanseri Türkiye de erkeklerde en sık görülen ürogenital sistem kanseridir. Tüm kanser türleri arasında ise akciğer kanserinden sonra ikinci sırada yer almaktadır. Radyoterapi prostat kanseri tedavisinde kullanılan yöntemlerden biridir. Ancak prostat kanseri hücreleri henüz tam olarak açıklanamayan nedenlerle radyoterapiye karşı direnç oluşturur. Bu çalışmada radyoterapinin, EAG1 potasyum kanal iletkenliği üzerine etkisinin incelenmesi amaçlanmıştır.

Yöntemler: Çalışmada DU145 prostat kanseri hücre hattı kullanılmıştır. Hücreler kontrol ve radyoterapi (RT) olarak iki gruba ayrılmıştır. RT grubundaki hücrelere tek doz 6 Gy RT uygulanmıştır. Kontrol grubundaki hücrelere herhangi bir uygulama yapılmamıştır. Uygulamadan 120 dakika sonra her iki grup için "tüm hücre patch-clamp tekniği" kullanılarak EAG1 kanal akımları kayıtlanmıştır. Daha sonra her bir kayıt için akım-voltaj eğrileri çizdirilerek eğriler bir doğru denkleminde uydurulmuştur. Doğrunun eğimi hesaplanarak kanal iletkenliği elde edilmiştir.

Bulgular: Kanal iletkenliği açısından kontrol grubu ile RT grubu arasında istatistiksel olarak önemli bir fark bulunmamıştır.

Sonuç: Sonuç olarak 6 Gy dozundaki RT'nin kanserli hücrelerde yüksek düzeylerde eksprese edilen ve kanser hücre proliferasyonu ile migrasyonunda önemli rol oynayan EAG1 kanal akımları üzerine etkisinin olmadığı gözlenmiştir.

Anahtar Sözcükler: Prostat kanseri, radyoterapi, patch-clamp, onkokanallar, EAG1 kanalları

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INTRODUCTION

Prostate cancer is one of the most common cancer types in the world. While ranking second among diseases that cause death in Europe and America, statistical analysis indicate an increase in the incidence of prostate cancer in Asia. (1, 2). Prostate cancer incidence has been reported 29 in the World and 42 in Turkey per 100 000 population (3). Although the causes of prostate cancer are not fully known, the factors affecting its incidence are well defined (4). Family history, age, race and genetic factors are the leading risk factors. In addition, obesity, diet and some environmental factors have been reported to play an important role in the development of prostate cancer (4).

Nowadays, many treatment methods are applied for prostate cancer. Although the treatment to be applied varies according to the stage of the disease, in general, surgery (radical prostatectomy), radiotherapy (RT) and hormonal treatment approaches are used alone or together according to the risk groups (5). In prostate cancer, RT can be given as external beam therapy, brachytherapy method known as placing radioactive sources directly in the prostate tissue, or combination of external beam therapy and brachytherapy (6). For RT, at 1.8-2 Gy fraction dose, all pelvis irradiation is done up to 45-50 Gy dose, and then up to 66-70 Gy dose with small area RT. (7).

RT in the treatment of prostate cancer is advantageous in that it is less invasive and better tolerated than surgical interventions. But prostate cancer cells create resistance to radiotherapy for reasons not yet fully explained (8). In several studies, this resistance has been associated with RT's ability to remove DNA damage in cancer cells, change the tumor microenvironment positively, increase the number of cell receptors of antioxidant enzymes, and inactivate free radicals caused by radiation by increased antioxidant activity (8, 9).

Ion channels are protein passages that ions use to cross the cell membrane. Ion channels are involved in many physiological processes that are important in both normal cell and cancer cell, such as cell volume regulation, cell migration, proliferation, death, and cell cycle (10). In recent studies, it has been reported that ion channels can play an important role in cancer development by increasing cell proliferation (10, 11, 12). Voltage sensitive ion channels form an important type of ion channels. These channels are either open or closed due to changes in the membrane potential (13). Voltage-sensitive K⁺ channels are responsible for maintaining the resting membrane potential in the cell. From the voltage sensitive potassium channel family, ether à-go-go potassium channels (EAG 1, Kv10.1) are expressed in cancerous cells rather than normal cells and play a role in the proliferation of cancerous cells (10). Studies showing that the EAG1 channel plays a role in cell cycle and cell proliferation in cancer tissue reveal the high oncogenic potential of this channel. (14).

It has been reported that ionized radiation changes ion transports in the cell membrane in RT treated cancer cells and this change is dose-dependent (15, 16). Changes in ion transport affect many cellular motor functions, including changes in cell volume and cytoskeleton. (17, 18). Although the expression of EAG1 channels in prostate cancer cells has been demonstrated in previous studies, no study investigating the effect of RT on EAG1 channel conductivity has been found. In this preliminary study, it was aimed to investigate the effect of single dose RT on EAG1 channel conductivity.

MATERIAL and METHOD

Cell Culture

DU-145 prostate cancer cells were used in the study (ATCC; Manassas, VA, USA). Cells were kept in an incubator which was set to 37 °C temperature, pH value in the range of 7.0-7.4 and a constant carbon dioxide (CO₂) amount at 5%. Cells were cultured in a medium that includes 100 mL RPMI (Roswell Park Memorial Institute medium) medium, 10 mL fetal bovine serum, 2.5 mL L-glutamine, 1 mL Penicillin-Streptomycin and 1 mL Amphotericin. After cultivation, the cells kept in the incubator were fed every 3-4 days until confluent.

Confluent cells to separate them from their containers for use in the study and passag them into other containers first washed three times with phosphate buffer solution (PBS), then incubated with trypsin ethylenediamine tetra acetic acid (EDTA) for 5 minutes (min.) and finally passaged into containers in a ratio of 1: 3.

Experiment Protocol and RT Application

The cells are separated into two groups as the control group and the RT group. No application has been made to the prostate cancer cells in the control group. Cells in the RT group received a single dose of RT. Since commercial culture cells were used in the study Ethics committee approval was not taken. In the study, a linear accelerator device (Siemens, PRIMUSTM, Germany) was used for irradiation of the cells in the RT group and the cells were exposed to 6 Gy radiation with 8 MeV electron energy as a single fraction with a dose rate of 300 MU / min. (Figure 1). Compared with photon energy, electron energy was used in irradiation due to the low accumulation of energy in the electron. A 5 mm bolus was placed on the petri plate in order to obtain a uniform dose distribution.

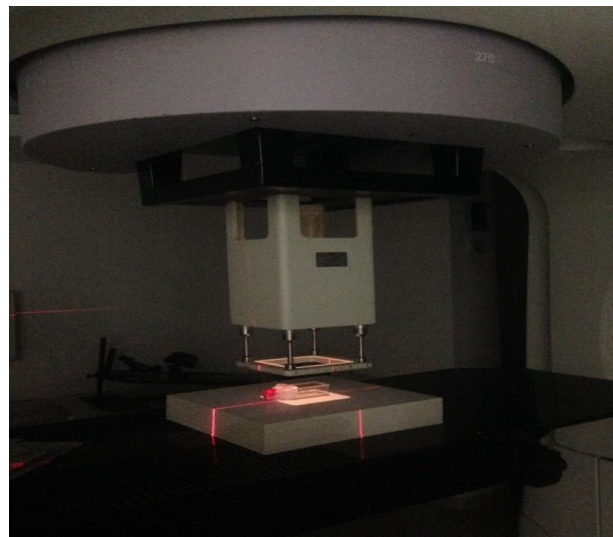


Figure 1. Radiotherapy application to DU145 cells.

Measurement of EAG1 Channel Conductivity with the Patch-Clamp Technique

By using the Multiclamp 700B patch-clamp amplifier (Axon Instruments, CA, USA) to detect EAG1 channel conductivity, current recordings were obtained in whole cell mode for both experimental and RT groups. The experimental setup is shown in Figure 2. The analysis of the records was done with Clampfit 11 software (Axon Instruments, CA, USA). The borosilicate glass pipettes used were pulled with a horizontal pipette hammer (Sutter Instruments Co. P-97) and made suitable for the records.

In the recordings, a bath solution with a content of 135 mM NaCl, 5mM KCl, 1mM CaCl₂, 1mM MgCl₂, 5 mM glucose 10 mM 4- (2-hydroxyethyl) -1-piperazineethanesulfonic acid (HEPES) (pH 7.2) and 150 mM KCl, 1 mM MgCl₂ and pipette solutions with 5 mM ethylene glycol tetraacetic acid (EGTA) and 10 mM HEPES (pH 7.2) were used.

All recordings were taken at room temperature (23 - 25 °C). After the cell membrane was clamped to the potential of -40 mV, currents were recorded at potentials between -100 mV and +100 mV in 20 mV steps. Four replicates (n = 4) were performed for each group. Reported electrophysiological *in vitro* data on irradiated tumor cells indicate that radiation-related transport changes may occur immediately and last up to 24 hours after irradiation (15, 19, 20). In the present study, similar to previous studies, channel currents were recorded 120 minutes after radiotherapy application.

In order to calculate conductivity, current-voltage curves were obtained by using Clampfit 11 analysis program. These curves were then fitted to the line equation with the Clampfit 11 analysis program. The line equation is given by

$$f(x)=mx+b$$

where m is the slope of the current-voltage curve ($\Delta I/\Delta V$) and corresponds to the channel conductivity.

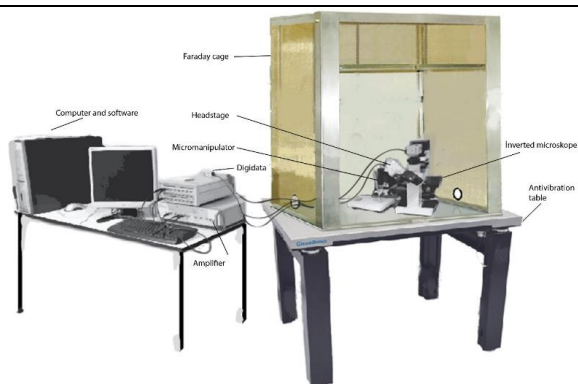


Figure 2. Whole cell patch-clamp recording setup.

Statistical Analysis

The data were analyzed by using IBM SPSS 20 (IBM, Istanbul) package program. The compatibility of conductivity values to normal distribution was shown with the Kolmogorov-Smirnov test, and the difference between the groups was tested with the student-t test. The limit of statistical significance was set at $p < 0.05$.

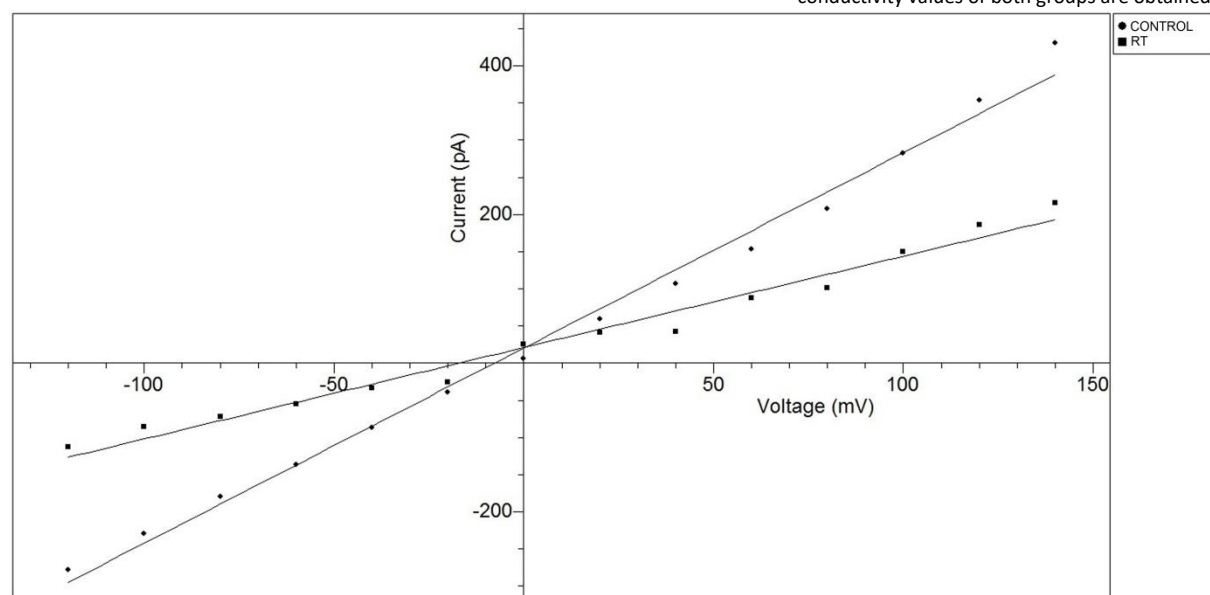


Figure 4. Current-voltage curve obtained from the groups and fitted the line equation.

While EAG1 channel conductivity was 1.29 ± 0.98 nS in the control group, this value was found to be 0.84 ± 0.20 nS in the RT group (Figure 5). No statistically significant difference was observed between the groups in terms of EAG1 channel conductivity ($p = 0.362$).

RESULTS

Figure 3A shows the voltage protocol applied to the cells, while 3B shows an sample of the corresponding current records.

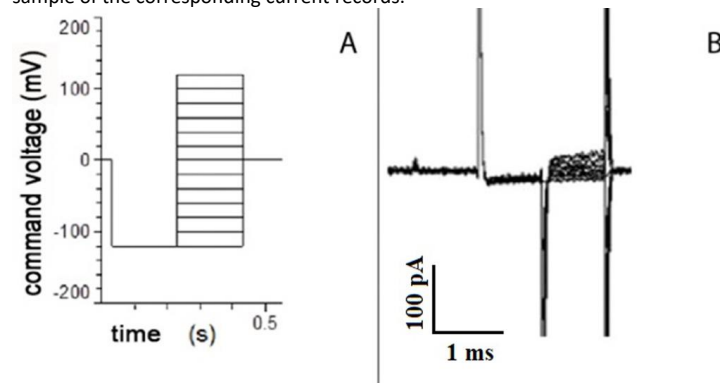


Figure 3. The voltage protocol (A) and an sample of current records applied to cells (B).

An example of the current-voltage curves obtained by using these voltage and current values in the control and experiment groups are given in Figure 4. By fitting these curves to a line equation, the slope of this line is calculated and conductivity values of both groups are obtained.

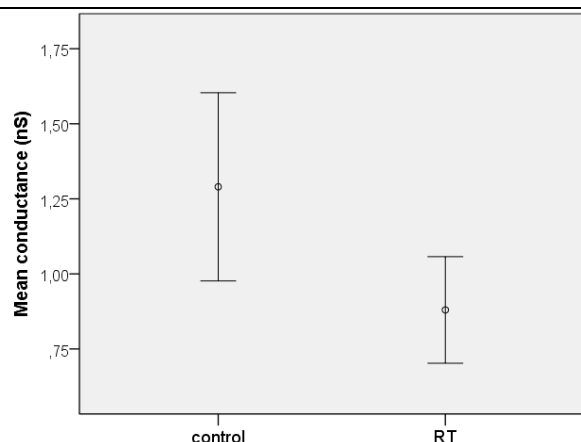


Figure 5. Mean channel conductivity values of control and RT groups.

DISCUSSION

Ion channels can play a role in the formation of mechanisms such as cell proliferation and apoptosis, as well as maintaining ion transport and membrane potential at normal values in cells. In particular, cancer cells express a number of different ion channels than their normal cells. These channels perform unique oncogenic functions in neoplastic transformation, tissue invasion and metastasis (21). In addition, they contribute to the formation of cellular stress response and radioresistance (22). One of the ion channels with high oncogenic potential is EAG1 channels. Blocking EAG1 channels has been reported to reduce tumor growth and development in various types of cancer (10). In this study, the effect of radiotherapy on EAG1 channel conductivity was investigated using the whole cell patch clamp technique and it was observed that radiotherapy did not have a significant effect on EAG1 channel conductivity. There are several studies examining the effect of ionizing radiation on ion channels. In one of these studies, Roth et al. showed that 0.1 Gy ionized radiation in epithelial lung cancer cell culture increased channel conductivity in two different potassium channels that are rectifying and self-activating (23). In another study conducted by Kuo et al. in human lung adenocarcinoma cells (A549), they observed a dose-dependent increase in K^+ currents in response to ionizing radiation. The increase in potassium currents started to appear at very small doses (10 cGy) and continued up to 150 cGy. While the dose-current relationship was linear between 10-150 cGy, it reached a maximum in 150 cGy and no significant change in K^+ channel current was observed at doses above 150 cGy. In the study, time-dependent changes in the K^+ current of 150 cGy dose were also observed (15). In a study by Steinle et al. in glioblastoma cells, it was reported that ionizing radiation in the 0.5-2 Gy dose range increased activation of large conductive potassium channels (16). Gibhardt, on the other hand, reported an increase in the conductivity of calcium-activated potassium channels of ionized radiation at a dose of 1 Gy in the study in human embryonic kidney culture cells (24). The earliest physiological response to the increase in potassium channel conductivities is hyperpolarization of the membrane potential (25). There is an important relationship between membrane potential and cell differentiation and cell proliferation, and membrane potential varies throughout the cell cycle (26, 27). These changes are hyperpolarization in the G1 / S transition in the cell cycle and depolarization in the G2 / M transition, and the source of changes in the membrane potential throughout the cell cycle is the change of ion channel activities (28). Increased potassium channel conductivity shows proliferative and suppression shows antiproliferative effect (29).

The results of these studies differ from the results of our study. This difference is thought to be related to the dose administered. Doses used in previous studies ranged from 0.1 Gy-2 Gy. These doses are lower than the 6 Gy dose used in this study. Similar to our study, studies using high-dose ionized radiation (5-100 Gy) reported that ionized radiation at these doses did not cause any membrane-related changes (30).

In addition, studies have shown that the proliferation and migration of cancer cells associated with an increase in potassium channel conductivity varies depending on the radiation dose used, low dose RT stimulates cell proliferation and migration by increasing calcium-activated potassium channel conductivity in human lung adenocarcinoma cells, and does not induce apoptosis (23, 31). On the other hand, it has been suggested that high dose reduces migration and invasion (31). These findings support the results of this study using a relatively high dose.

Studies reporting that K^+ channel conductivity and consequently increasing cell proliferation by using low-dose ionizing radiation in cancer cells are important in terms of demonstrating that low-dose ionizing radiation positively affects tumor growth. This effect may be related to the resistance to radiotherapy in some types of cancer. However, as in this study, higher doses of radiotherapy may prevent the development of resistance and tumor growth by not changing the conductivity of oncochannels or reducing conductivity. Findings in the literature support the idea that resistance to radiotherapy is related to the dose administered. The most important limitation of this study is that the EAG1 channel flow was measured for single dose radiotherapy only. To eliminate this, it would be useful to examine the subject in a wider dose range and to show changes in channel expression in addition to conductivity.

Consequently, in this study, administration of single dose 6 Gy RT to DU145 prostate cancer cells decreased the channel conductivity by approximately 32% in RT treated cells, but this decrease was not found statistically significant. Although this result suggests that the proliferation of cancer cells can be prevented by using higher doses of RT in DU145 cells, considering the risks that high-dose RT may pose in healthy cells, it is thought that using EAG1 channel blockers as an alternative to RT may be less risky. However, further studies are needed in this regard.

Conflict of interest

No conflict of interest was declared by the authors.

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