Journal of Physical Chemistry and Functional Materials

Home Page of Journal: https://dergipark.org.tr/jphcfum

Effect of Electroporation on Radiotherapy Treatment in Human Hepatocellular Carcinoma Cells

*Mehmet Eşref Alkış**, *Sefa Yeşilbaş*

AlparslanUniversity, Muş, Turkey
** Corresponding author: Mehmet Eşref ALKIŞ (E-mail: me.alkis@alparslan.edu.tr)*

ABSTRACT

Thanks to technological developments and clinical studies in recent years, radiotherapy has been widely used in cancer treatment, and radiation can be applied effectively to cancer cells without harming healthy tissues. However, some types of cancer cells are resistant to radiotherapy which can be applied at certain doses that do not harm normal tissues. In this context, the main purpose of this study is to increase the sensitivity of cancerous cells to radiotherapy and by doing so to reduce the negative side effects of radiotherapy with lower doses of radiation and to get more efficient results through the combined use of treatments. In this study, HepG2 (Human hepatocellular carcinoma) liver cancer cells were treated by ionizing radiation (210 kV X-ray at a dose rate of 2.1 Gy/min) or electroporation (1125 V/cm, 100 µs, 1 Hz, 8 square waves) or combination of these two therapies. Responses to these treatments were determined by the MTT viability test. It was observed that the survival rate of HepG2 cancer cells significantly decreased in the group treated with ionizing radiation after electroporation. The electrical pulses caused a 1.25-fold increase in the sensitivity of HepG-2 cancer cells to 210 kV X-ray. These results show that the application of electroporation before radiotherapy can significantly increase the sensitivity of HepG2 cancer cells.

ARTICLE INFO

Keywords:

Electroporation
HepG2
Ionizing radiation
Radiotherapy

Received: 08-10-2021
Accepted: 14-11-2021
ISSN: 2651-3080
DOI: 10.54565/jphcfum.1006281

1. Introduction

Cancer is one of the most important diseases that mankind has struggled with in the last century. It has spread rapidly and become the second cause of death worldwide after heart attack. 18.1 million new cancer cases and 9.6 million cancer-related deaths were recorded across the world in 2018 [1]. Liver cancer is the 6th most common cancer and the 4th deadliest cancer [2]. 841,000 new liver cancer cases and 782,000 liver cancer related-deaths were recorded worldwide in 2018 [3].

As with all other types of cancer, radiotherapy and chemotherapy are the 2 most common methods used in the treatment of liver cancer [4]. Radiotherapy is used effectively in more than 60% of cancer patients. Thanks to the technological developments in recent years, the size and location of cancerous tumors can be identified clearly, and this makes it possible to adjust the dose of radiotherapy in a way not to damage the surrounding disease-free tissues [5]. For example, 20 years ago, the death rate from cancer radiation therapy was 80% in head and neck cancers, but in recent years this rate has decreased to 30% [6]. However, some types of liver cancer cells are resistant to radiotherapy which can be applied at certain doses that do not harm normal tissues. In such cases, combined treatments can be used to increase the radiation sensitivity of these cells [6]. Combined use of radiotherapy and electroporation is one of these methods [7].

Electroporation is a process where the cell membrane is made more permeable to exogenous molecules by a local exposure to a high voltage electric current [8]. Electroporation technique makes it possible to transfer chemotherapeutic drugs, protein or DNA through the cell membrane [7]. The most important factors affecting the effectiveness of electroporation are as follows: intensity and duration of the applied current, type and size of cell [9]. Moreover, the electroporation conditions required for different molecules are also different. For example, a short and intense current is needed for small-sized cancer drugs, but a long and low-intensity current for gene transfer to the cell [10]. Studies have shown that electroporation is more effective in cancer cells than in healthy ones [11]. Previous studies have reported that electroporation in different protocols increases the sensitivity of cancer cells to radiotherapy [7].

Electroporation also leads to the production of reactive oxygen species (ROS) in the cell membrane [12]. ROS can

sensitize cells to ionizing radiation. To the best of our knowledge, in the literature, there is no study examining how electroporation affects the effectiveness of radiotherapy on liver cancer cells. The purpose of this study was to examine the effectiveness of the application of electroporation prior to ionizing radiation in HepG2 liver cancer cell line. We used HepG2 because it is more advantageous in terms of ease of study and cell culture than other cell lines and widely used in many pharmacological studies [13].

2. Materials and Methods

2.1. Cell Culture

The HepG2 (human hepatocellular carcinoma) cell line used in this study was procured from Application and Research Center, Central Research Laboratories, Muş Alparslan University. The cells were cultured in Dulbecco's Modified Eagle's medium (DMEM) (Biological Industries) containing 10% fetal bovine serum (FBS) [PAN-Biotech, Europe] and 1% penicillin-streptomycin (Sigma-USA) using 75 cm^2 culture flasks (Sigma-USA). Then, the cells were incubated in DMEM with 5% CO_2 at 37^oC and 95% humidity (Memmert-Germany). The old medium was replaced by a new one every two days. After the incubation, when 90% of the bottom of flasks was covered with cells, the cells were included in the experiment. All the cell culture procedures were carried out in a biosafety cabinet (Esco-USA) under sterile conditions. The cells were divided into 4 groups: control group, EP group (electroporation alone), IR group (ionizing radiation alone), and EP+IR group (first electroporation and then ionizing radiation).

2.2. Electroporation (EP)

After the incubation, the cells that reached sufficient density on the flask surfaces were removed from the flask using Trypsin-EDTA (Biological Industries, Israel). Then, this cell solution was placed in falcon tubes and centrifuged at 1200 rpm for 5 minutes. 400 µL of cell suspension ($1x10^6$ cells/mL) was transferred to 4 mm EP cuvettes (BTX). The cuvettes in EP group were placed in an electroporation device (Gemini X2, BTX, USA) to apply electric field. Based on the results of our previous studies and others, we determined that 1125 V/cm was the most suitable electric field in EP application for the HepG2 cell line [14, 15]. Again, based on these studies, 8 square wave electric fields were applied (1125 V/cm, 100 µs, and 1 Hz). The cells in EP+IR group were exposed to ionizing radiation 10 minutes after EP. The control cells prepared in the same way were placed in these cuvettes, but no electric field was applied to them. This experiment was repeated 5 times.

2.3. Radiation Therapy (IR)

10 minutes after the electroporation [7], the cells in EP+IR group were exposed to X-ray using BMX-AR 30 (BMI, Italy) at room temperature. 210 kV X-ray radiation was applied to the cells at an intensity of 2.1 Gy/min [8]. During the application, a plexiglass with a size of 10x10 cm and a thickness of 1.5 mm was placed on the top of the cell cuvettes and another plexiglass with the same size and a thickness of 3 mm was placed below their bottom to ensure backscattering of the radiation. About 15 minutes after the electroporation and ionizing radiation application, all the experimental cells were seeded into 96-well plates with 10,000 cells for each well. The plates were incubated for 24 hours under 5% CO_2 at 37^oC and 95% humidity, and then the cell viability was determined using MTT assay method.

2.4. MTT Cell Viability Assay

MTT (3-[4,5-dimethylthiazol-2-yl]-2,5diphenyltetrazolium bromide) cell viability test is a sensitive method widely used in many studies to determine cell viability [16]. This method is based on the logic that the dehydrogenase enzyme in the mitochondria of viable and healthy cells can cleave tetrazolium rings in the MTT dye under in vitro conditions. Viable cells reacting in this way are stained blue, while dead and deteriorated ones remain colorless [17]. First, the MTT solution with a concentration of 5mg/mL was covered with aluminum foil and kept at +4 °C until use. Then, the cell lines incubated for 24 hours in 96-well plates were removed from the incubation. The cell line medium in the plates was removed from the wells, and then 10 µL of MTT solution and 90 µL of DMEM were added to the wells. These plates were incubated at 37 ^oC under 5% CO_2 for 4 hours. After the incubation, the liquids containing MTT in the wells were removed by aspiration, and 100 µL of Dimethylsulfoxide (DMSO) was added to dissolve formazan crystals for quantitative analysis. After the complete dissolution of formazan crystals and the formation of a blue color, the optical density of the formazan was read using an optical reader at 570 nm [15, 18]. The absorbance values of the wells of the control group, in which only fresh medium was used, were measured, and the measured values were averaged. This value was accepted as 100% viable cell ratio. The absorbance values of the cell lines in all groups except the control group were measured, and the viability ratio (%) was calculated using the following equation.

Viability (%) = (Optical Density of Study Group / Optical Density of Control Group) x 100

2.5. Statistical Analysis

All the experiments were repeated at least 3 times, and all the data were expressed in mean ± standard deviation. The differences between the groups in terms of cell amounts were identified using one-way analysis of variance (ANOVA), and multiple comparisons were performed using the Tukey's multiple comparison test. The statistical significance was set at $p<0.05$.

3. Results

In this study, the effects of electroporation applied to the HepG2 cell line, which is widely used in many studies on liver cancer, on the efficiency of radiotherapy were

examined. Electroporation was applied alone to the first cell group, and ionizing radiation alone to the second cell group. In the third cell group, these two methods were combined, and ionizing radiation was applied after electroporation. As can be seen in Figure 1, it was observed that there were significant differences in terms of cell viability between the cells treated with electroporation and ionizing radiation in a combined way and those treated with electroporation alone or ionizing radiation alone. While the cell viability ratio was found to be 88.74% in the group "electroporation alone", it was 80.52% in the group "ionizing radiation alone." On the other hand, 64.5% of the cells survived in the combined use of electroporation and ionizing radiation.

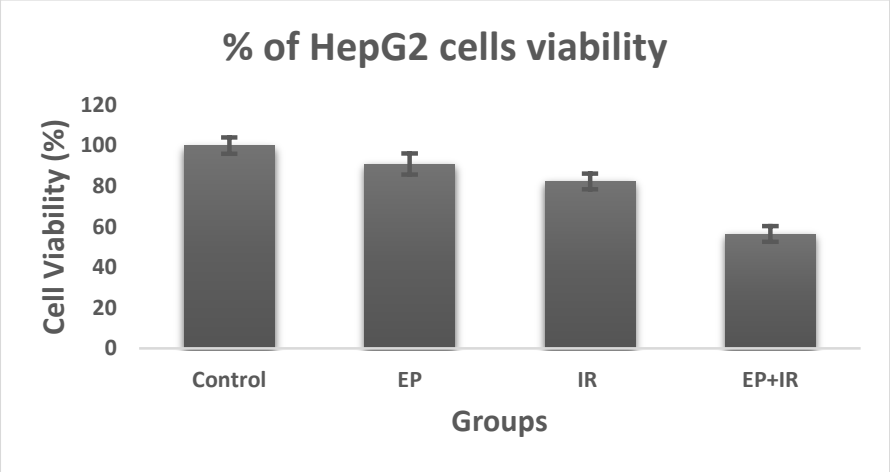

Fig. 1. % of the HepG2 cell viability for treatment by ionizing radiation alone (IR), electroporation alone (EP) and, EP application prior to IR (EP+IR). Viability percentages of HepG2 cells are given as the mean±SD of at least three different experiments.

These results suggest that application of electroporation prior to radiotherapy can significantly improve the efficiency of radiotherapy in the treatment of HepG2 liver cancer cells.

4. Discussion

Liver cancer, one of the most common and deadly cancers, claims thousands of lives across the world every year [19]. Today, radiotherapy is one of the leading treatment options for patients with liver cancer [20]. The effectiveness and efficiency of radiotherapy depend on the radiation sensitivity of the tumor and the radiotherapy tolerance of the surrounding healthy liver tissue [21]. Therefore, the main subject that needs to be examined is how to increase the sensitivity of cancer cells to radiotherapy and by doing so to reduce possible side effects and to obtain more efficient results with lower doses of radiation.

In this study, it was observed that the cancer cells were sensitive to ionizing radiation, and electroporation significantly increased this sensitivity ($p<0.05$). After the treatments, the viability of HepG2 cancer cells were found to be 88.74% in the EP group, 80.52% in the IR group, and 64.5% in the EP+IR group. These results are in line with those reported by previous similar studies. In their study on Chinese hamster ovary (CHO) cells, Razaee et al. [22] compared the viability ratios of the cancer cells treated with radiotherapy alone and those treated with electroporation and radiotherapy together. They reported that while the viability ratio was 76.73% in the group treated with radiotherapy alone, it was 65.1% in the group treated with electroporation 10 minutes before the radiotherapy. In another study on Ehrlich acid tumors (EAT), it was observed that, in the radiotherapy following electroporation therapy, various reactive oxygen species (ROS) were produced in cancer cells and the cell toxicity increased with the resulting oxidative stress. It was asserted that this increase caused more damage to the cell membrane. On the 7th day of the treatment, the mean tumor volumes were reported to decrease by 85%, 80%, and 51% compared to the pre-treatment sizes in the electroporation group, radiation group, and electroporation + radiation group, respectively. These results showed that the electric field significantly increased the radiation sensitivity and drug-induced toxicity [23]. The possible reason why electroporation increases the effect of ionizing radiation is the production of ROS in the membrane region where electric pulses are applied [12] and the changes in the membrane.

There are also some studies on various treatment methods in which electroporation is combined with drugs that enhance radiation sensitivity. Serša et al. [8] applied a 3-stage treatment method on Ehrlich acid tumors (EAT) in mice using Cisplatin, a drug that is widely used in chemotherapy and has an effect of enhancing radiation sensitivity. First, they injected cisplatin into the organism and then applied an electric field to the target tumor, followed by local radiation therapy to the tumor. They reported that this 3-stage method was more efficient than the 2-stage method in which cisplatin and radiation therapy is applied alone, and the curability rate of EAT increased from 27% to 92%. This increase in anti-tumor activity is thought to be associated with the electric field applied. Similarly, Kranj et al. [24] used Bleomycin, a drug that has an effect of enhancing radiation sensitivity, in their study on soft tissue cancer cells. They reported that electroporation prior to drug administration caused a 1.5-fold increase in the radiotherapy sensitivity of cancer cells. It is thought that electroporation increases the effectiveness of drugs by increasing the permeability of the cell membrane and the accumulation of drugs in the cells [25, 26].

As known, the main target of therapeutic radiation is the DNA of tumor cells. The objective is to cause cell death by damaging the target cell DNA using different types of radiation. The biggest negative side effect in this process is that healthy cells and tissues surrounding tumor are also affected by radiation. This mechanism of action can cause permanent damage to DNA of healthy cells and lead to undesired chemical reactions by triggering the production of some free radicals [27].

5. Conclusion

In this study, we aimed to get more efficient results with lower doses of ionizing radiation by using electroporation to increase the radiation sensitivity of the cells, and we were able to achieve supporting results. Our results suggest that electroporation can increase the radiation sensitivity of HepG2 liver cancer cells. Electroporation can be considered as a physical radiosensitizer, especially in the radiotherapy treatment of radioresistant cancer cells. Applicability of this combined therapy method against different cancer cells and tumors should be examined and assessed in clinical settings. We hope that the results of this study will contribute to other studies on electroporation and radiotherapy in cancer treatment.

References

[1] M.C. Hulvat, Cancer incidence and trends, *Surgical Clinics,* 2020, **100**, 469-481.

[2] P. Dasgupta, C. Henshaw, D.R Youlden, P.J. Clark, J.F. Aitken, P.D. Baade, Global Trends in Incidence Rates of Primary Adult Liver Cancers: A Systematic Review and Meta-Analysis, *Frontiers in oncology*, 2020, **10,** 171.

[3] F. Bray, J. Ferlay, I. Soerjomataram, R.L. Siegel, L.A. Torre, A. Jemal, *Erratum:* Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries, *CA Cancer J Clin,* 2020, **70**, 313.

[4] Q. Zhang, F.X. Wang, K. Jia, L. Kong, Natural product interventions for chemotherapy and radiotherapy-induced side effects, *Frontiers in pharmacology*, 2018, **9,** 1253.

[5] M. Baumann, M. Krause, J. Overgaard, J. Debus, S.M. Bentzen, J. Daartz, C. Richter, D. Zips, T. Bortfeld, Radiation oncology in the era of precision medicine, *Nature Reviews Cancer*, 2016, **16,** 234-249.

[6] H.H. Chen and M.T. Kuo, Improving radiotherapy in cancer treatment: Promises and challenges, *Oncotarget*, 2017, **8,** 62742-62758.

[7] A. Yadollahpour, Z. Rezaee, V. Bayati, M.J.T. Birgani, and F.N. Dehbashi, Radiotherapy enhancement with electroporation in human intestinal colon cancer HT-29 cells*, Asian Pacific journal of cancer prevention: APJCP*, 2018, **19,** 1259-1262.

[8] G. Serša, S. Kranjc, and M. Čemažar, Improvement of combined modality therapy with cisplatin and radiation using electroporation of tumors, *International Journal of Radiation Oncology* Biology* Physics*, 2000, **46,** 1037-1041.

[9] J. Gehl, Electroporation: theory and methods, perspectives for drug delivery, gene therapy and research, *Acta Physiologica Scandinavica*, 2003, **177,** 437-447.

[10] D. Rabussay, N.B. Dev, J. Fewell, L.C. Smith, G. Widera, and L. Zhang, Enhancement of therapeutic drug and DNA delivery into cells by electroporation*, Journal of Physics D: Applied Physics*, 2003, **36,** 348-363.

[11] M.E. Alkış, Ü. Keleştemür, Y. Alan, N. Turan, and K. Buldurun, Cobalt and ruthenium complexes with pyrimidine based schiff base: Synthesis, characterization, anticancer activities and electrochemotherapy efficiency, *Journal of Molecular Structure*, 2021, **1226,** 129402.

[12] B. Gabriel, and J. Teissie, Generation of reactive-oxygen species induced by electropermeabilization of Chinese hamster ovary cells and their consequence on cell viability, *European Journal of Biochemistry*, 1994, **223,** 25-33.

[13] M. Cemazar, I. Hreljac, G. Sersa, and M. Filipic, Construction of EGFP expressing HepG2 cell line using electroporation, in IFMBE Proceedings, Berlin, Heidelberg: Springer Berlin Heidelberg, 2009, 128–131.

[14] L.G. Campana, D. Miklavčič, G. Bertino, R. Marconato, S. Valpione, I. Imarisio, M.V. Dieci, E. Granziera, M. Cemazar, and M. Alaibac, Electrochemotherapy of superficial tumors–Current status: Basic principles, operating procedures, shared indications, and emerging applications, *Semin. Oncol*, 2019, **46**, 173–191.

[15] M.E. Alkış, K. Buldurun, N. Turan, Y. Alan, Ü.K. Yılmaz, and A. Mantarcı, Synthesis, characterization, antiproliferative of pyrimidine based ligand and its Ni (II) and Pd (II) complexes and effectiveness of electroporation, *Journal of Biomolecular Structure Dynamics*, 2020, 1-11.

[16] E. Grela, J. Kozłowska, and A. Grabowiecka, Current methodology of MTT assay in bacteria–A review, *Acta histochemica,* 2018, **120,** 303-311.

[17] A.B. Yıldırım, E. Mutlu, and M. Yıldırım, Cytotoxic Effects of Thiazolo [3, 2-C] Pyrimidines Against Mcf-7 And Hepg2/C3a Carcinoma Cell Lines, *Hacettepe Journal of Biology Chemistry*, 2018, **46,** 237-246.

[18] P. Kumar, A. Nagarajan, and P.D. Uchil, Analysis of cell viability by the MTT assay, *Cold Spring Harbor Protocols*, 2018, **2018,** pdb. prot095505.

[19] K. Hara, A. Takeda, Y. Tsurugai, Y. Saigusa, N. Sanuki, T. Eriguchi, S. Maeda, K. Tanaka, and K. Numata, Radiotherapy for hepatocellular carcinoma results in comparable survival to radiofrequency ablation: a propensity score analysis*, Hepatology*, 2019, **69,** 2533-2545.

[20] C. Choi, G. S. Yoo, W. K. Cho, and H. C. Park, Optimizing radiotherapy with immune checkpoint blockade in hepatocellular carcinoma, *World journal of gastroenterology*, 2019, **25,** 2416-2429.

[21] Y. H. Lee, D. Tai, C. Yip, S. P. Choo, and V. Chew, Combinational Immunotherapy for Hepatocellular Carcinoma: Radiotherapy, Immune Checkpoint Blockade and Beyond, *Frontiers in Immunology*, 2020, **11,** 568759.

[22] Z. Rezaee, A. Yadollahpour, and V. Bayati, Single Intense Microsecond Electric Pulse Induces Radiosensitization to Ionizing Radiation: Effects of Time Intervals Between Electric Pulse and Ionizing Irradiation*, Frontiers in oncology*, 2018, **8,** 418.

[23] P. Shil, P. Vidyasagar, S. Sanghvi, and K.P. Mishra, Enhancement of cytotoxic effects of radiation and drug by electroporation in cancer cells: in vitro and in vivo studies, *BARC Newslett*, 2006, **273,** 167-171.

[24] S. Kranjc, M. Cemazar, A. Grosel, M. Sentjurc, and G. Sersa, Radiosensitising effect of electrochemotherapy with bleomycin in LPB sarcoma cells and tumors in mice, *BMC cancer*, 2005, **5,** 115.

[25] M.E. Alkış, N. Turan, Y. Alan, S. Irtegun Kandemir, and K. Buldurun, Effects of electroporation on anticancer activity of 5-FU and newly synthesized zinc(II) complex in chemotherapy-resistance human brain tumor cells, *Med Oncol,* 2021, **38,** 129.

[26] M.E. Alkis, Effects of electroporation on cytotoxicity of 4-aminopyrimidin-2-(1H)-one based ligand and its Cobalt (II) and Ruthenium (II) complexes in MCF-7 cancer cells, *Dicle Med J*, 2021, **48,** 498–506.

[27] H. de A. Carvalho and R. C. Villar, Radiotherapy and immune response: the systemic effects of a local treatment*, Clinics*, 2018, **73,** e557s.