Original Article

# Effect of radiation dose rates and cisplatin on cytogenetic damage in rats receiving head–neck radiotherapy

**Dilek Unal, Aslihan Kiraz[1], Serhat Aydogan[2], Zeynep Soyer Sarica[3], Harun Celik[4], Ebru Akay[5], Celalettin Eroglu[6], Bunyamin Kaplan[4]**

Department of Radiation Oncology, Istanbul Medipol University, Faculty of Medicine, Istanbul, [1]Department of Medical Genetic, Kayseri Research and Education Hospital, Kayseri, [2]Department of Anesthesiology, Kayseri Education and Research Hospital, Kayseri, [3]Erciyes University Experimental Research and Application Center, Kayseri, [4]Department of Radiation Oncology, Acıbadem Kayseri Hospital, Kayseri, [5]Department of Pathology, Kayseri Education and Research Hospital, Kayseri, [6]Department of Radiation Oncology, Erciyes University Medical School, Kayseri, Turkey

**For correspondence:** Dr. Dilek Unal, Department of Radiation Oncology, Istanbul Medipol University, Faculty of Medicine, Istanbul, Turkey.
E-mail: dilekunaldr@gmail.com

## ABSTRACT

**Background:** We aimed to investigate effect of radiotherapy (RT) applications with different dose rates on cytogenetic damages, which focused on micronucleus (MN) formation, and evaluate how this damage varies by cisplatin in rats receiving head–neck RT.

**Material and Methods:** Thirty-six Sprague Dawley rats were divided into five groups. The first and second groups were irradiated at a dose rate of 300 monitor unit/minute (MU/min) and 600 MU/min, respectively. The third group was irradiated at a dose rate of 300 MU/min and given cisplatin. The fourth group was irradiated at a dose rate of 600 MU/min and given cisplatin. The fifth group received neither irradiation nor cisplatin (control group). One thousand polychromatic erythrocytes were scored, and MN frequency in polychromatic erythrocytes was determined for each rat.

**Results:** There was a significant difference among five groups in terms of the number of MN (*p*: 0.001). The number of MN was significantly higher in the 600 MU/min + cisplatin group (fourth group) compared to the control group [9.5 (1.0–23.0) vs. 1.5 (1.0–2.0), respectively]. It was also significantly higher in 600 MU/min + cisplatin group (fourth group) compared to 300 MU/min group (first group) [9.5 (1.0–23.0) vs. 2.0 (1.0–3.0), respectively]. On the other hand, there was no significant difference among other groups.

**Conclusions:** Our findings suggest that RT given at a higher dose rate causes more cytogenetic damage, and this damage is increased by concurrent administration of cisplatin.

**KEY WORDS:** Cisplatin, cytogenetic damage, micronucleus, radiotherapy dose rate, rat

## INTRODUCTION

Radiotherapy (RT) is one of the major treatment modalities for cancer patients. However, it affects normal tissues.[1] Ionizing radiation has clastogenic effects and carcinogenic potential. Deoxyribonucleic acid (DNA) is a principal target of ionizing radiation that can produce DNA damage of various types, including nucleotide base damage, DNA single-strand breaks, and double-strand breaks (DSBs), which are most accused for mutations and carcinogenesis. Unrepaired or misrepaired DSBs are considered to be a threat to genomic integrity.[2] RT can be given with different dose rates.[3] Dose rate means the duration of administration of a particular dose. It is one of the major factors that determine the degree of biological effect, which is generated by a certain dose of X and gamma radiation. A decrease in the dose rate with prolongation of irradiation time generally reduces the degree of resultant biological effect. The acute and chronic side effects of different RT dose rates are still investigating.

Cisplatin, a DNA-crosslinking agent, is a potent antineoplastic agent used in the treatment of a number of malignancies and is also used to be a radiosensitizer. It has cytotoxic effects on normal cells.[4,5] It is the most commonly used chemotherapy agent in head and neck cancers.

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Micronuclei are small extranuclear cytoplasmic bodies and derive from chromosomal fragments and whole chromosomes lagging behind in anaphase.[6] Increased micronucleus MN (frequency) is considered to be an indirect indicator of numerical and structural chromosome disturbances in cells caused by a large number of potentially genotoxic chemical agents and ionizing radiation.[6,7]

Head and neck cancers are the group of diseases for which RT is a curative treatment. Therefore, long-term survival is expected in patients with cancers, and the cytogenetic damage effect of RT can be mostly seen in the course of the disease.

MN formation is a valuable marker to determine the cytogenetic damage caused by RT, which can predict the development of secondary malignancy after RT. Advances in RT technology allow the application of RT with different dose rates. In our previous study, we found an increase in peripheral blood MN in head and neck cancer patients with long-term survival who were treated RT or chemoradiotherapy (CRT)[8]. In this experimental animal study, we aimed to investigate the effect of radiation dose rate on MN formation, which is a marker of cytogenetic damage, and evaluate how this damage varies by cisplatin in animals given irradiation to head and neck region.

## MATERIALS AND METHODS

### Animals

Thirty-six adult male Sprague Dawley rats, with a mean body weight of 302 ± 30 gr, were used in this study. Rats were housed in separate cages and given ad libitum access to food and water. Animals were acclimatized in a 12-h light/dark cycle. This study was approved by the Local Ethical Committee for Animal Studies (No: 14/165).

### Study design and animal experimentation

The rats were divided into five groups, each of which included eight rats, except the control group, in which there were four rats. Rats in the first group were irradiated at the dose rate of 300 monitor unit/minute (MU/min), whereas those in the second group were irradiated in the dose rate of 600 MU/min. Rats in the third group were irradiated at the dose rate of 300 MU/min and given cisplatin. Rats in the fourth group were irradiated at the dose rate of 600 MU/min and given cisplatin. The the fifth group was designated as the control group and received neither irradiation nor cisplatin.

RT was applied by 6 megavoltage (MV) X-ray external beams via a linear accelerator, Varian DHX 2300 (Varian Medical Systems, Stangenes Industries, Inc., Palo Alto, California, USA). The rats who underwent anesthesia were immobilized on the board before RT. The bottom of the eyeball was designated as the cranial margin of the head and neck RT field, whereas the upper part of the humeral head was designated as its caudal margin. Rats were laid on the ear, and all groups except the control group received irridation as a single fraction dose of 10 Gy.

In the third and fourth groups, a single dose of 5 mg/kg cisplatin (Cisplatin Ebewe®) was administered intraperitoneally just before RT application.

MN frequency in the polychromatic erythrocytes of femoral bone marrow was evaluated according to the procedure of Schmid.[9] The rats were sacrificed by cervical dislocation method under general anesthesia 72 h after RT application, and femurs of each rat were bilaterally harvested. The femoral bone marrow was transferred to the centrifuge tube by a syringe containing fetal serum. The examples were centrifuged at 1000 rpm for 5 min, and the supernatant was discarded.

Some fetal serum was put into the remaining portion and suspended. The suspension was spread on lam plates, which were stained using the May–Grunwald stain. The slides were examined by light microscope 40 × and 100 × magnification. One thousand polychromatic erythrocytes were scored, and MN frequency in the polychromatic erythrocytes was determined for each rat.[7,10]

### Statistical analysis

SPSS 22.0 statistic software was used for the statistical analysis. The Shapiro–Wilk test was performed to assess the assumption of normality of the variables. Because the variables, number of MN, were not distributed normally, they were presented as median (minimum-maximum). Statistical analysis for nonparametric variables was performed by Kruskal–Wallis test followed by Dunn's Bonferroni post hoc test for multiple comparisons. A $P$ value of $<0.05$ was considered to be significant.

## RESULTS

Table 1 shows a comparison of the number of MN in 1000 polychromatic erythrocytes among experiment groups. There was a significant difference among experiment groups in terms of the number of MN ($p$: 0.001). The number of MN was significantly higher in the 600 MU/min + cisplatin group (fourth group) compared to the control group [9.5 (1.0–23.0) vs. 1.5 (1.0–2.0), respectively]. It was also significantly higher in the 600 MU/min + cisplatin group (fourth group) compared to 300 MU/min group (first group) [9.5 (1.0–23.0) vs. 2.0 (1.0–3.0), respectively]. On the other hand, there was no significant difference among other groups. However, the number of MN in the 600 MU/min group (second group) was the second highest value [4.0 (2.0–9.0)].

## DISCUSSION

In this experimental study, we found that irradiation with a high dose rate caused more cytogenetic damage, and this damage is further increased by cisplatin.

**Table 1: Comparison of the number of micronuclei in 1000 polychromatic erythrocytes among experiment groups**

| | 300 MU/min[b] *n*=8 | 600 MU/min *n*=8 | 300 MU/min + cisplatin *n*=8 | 600 MU/min + cisplatin[a,b] *n*=8 | Control[a] *n*=4 | *P* |
|---|---|---|---|---|---|---|
| Number of micronuclei | 2.0 (1.0-3.0) | 4.0 (2.0-9.0) | 3.0 (2.0-8.0) | 9.5 (1.0-23.0) | 1.5 (1.0-2.0) | 0.001 |

[a]There was a significant difference between the 600 MU/min + cisplatin group and the control group. [b]There was a significant difference between the 600 MU/min + cisplatin group and the 300 MU/min group

Currently, RT can be applied with high dose rates. On the other hand, this leads to the question of whether radiobiological results may occur.[11] The radiobiological effects of different RT dose rates are still investigating. Sørensen *et al.* investigated the radiobiological effect of this high instantaneous dose rate on two cell lines, which were Chinese hamster lung fibroblasts and human head and neck squamous cell carcinoma, and found that there was no effect of the instantaneous dose rate on cell survival for both cell lines.[11] Hurmuz *et al.* evaluated the effect of different dose rates (a single dose of 12 Gy in dose rate of 300 and 600 MU/min) on rat lung tissue. They observed that dose rates might have an impact on normal tissue toxicity, and fibrosis was a striking effect of increased dose rate in rat lung tissue.[12] To the best of our knowledge, this is the first study, in which the effect of RT applications with different dose rates on cytogenetic damages was investigated. In our study, we found that the 600 MU/min + cisplatin group had the highest number of MN, whereas the 600 MU/min group had the second highest value.

Most chemical agents and different types of radiation have multiple effects at the molecular, cellular, and chromosomal level, which may occur simultaneously and to varying extents depending on the dose.[13] Micronuclei can originate from exposure to various clastogenic agents in the form of acentric chromosome fragments and aneugenic agents in the form of whole chromosomes. Ionizing radiation is a strong clastogenic agent, and thus it induces MN formation.[14] It has been shown that MN formation increases during RT in cancer patients. Jagetia *et al.* investigated how does MN frequency change during RT in patients suffering from various types of cancer and observed that there was a significant increase in MN frequency during the middle of RT in most of the patients when compared to the pretreatment samples. MN frequency further elevated immediately after the completion of RT, and this elevation was significantly higher than that of pretreatment and mid-treatment samples.[15] Similarly, in testicular seminoma patients treated with adjuvant RT after orchiectomy, Gamulin *et al.* observed that MN frequency had started to increase immediately after the onset of RT, and this had continued to increase in the course of therapy RT and reached the highest value at the end of RT.[16]

Radiation therapy in some patients induces a systemic antitumor response known as the abscopal effect. The radiation-induced abscopal effect has been defined as a reaction outside an irradiated field but within the same organism.[17] Radiation-induced abscopal effects can be either beneficial or detrimental.[18] The beneficial abscopal effect is a regression of the tumor outside the irradiated field after applying localized irradiation to a separate tumor location.[19,20] On the other hand, unirradiated cells and tissues are also damaged. The abscopal effects include induction of genomic instability, DNA damage, apoptosis, cell death, and oncogenic transformation in normal tissues.[18,21]

Siva *et al*. have investigated the response of normal tissues outside the irradiated field during curative RT in patients with nonsmall cell lung carcinoma. They have sampled eyebrow hairs, which is a surrogate model for out-of-field normal tissues, and found a significant increase in γ-H2AX foci, a biomarker of DNA damage underlying cellular response to irradiation, in eyebrow hair follicles 24 h after local irradiation therapy of a separate distant organ tumor and the increase remained high during treatment, in a dose-independent manner. In other words, delayed abscopal DNA damage response after irradiation has been observed in eyebrow hair follicles. They have concluded that localized RT induces pronounced systemic DNA damage in normal tissues.[22]

In the present study, we examined the number of MN formed in the bone marrow by applying the same dose of RT at different dose rates. Increased MN formation in the bone marrow after irradiation to the head and neck region can also be an indicator of the abscopal effect. In other words, applying RT with a high dose rate can create an abscopal effect and thus affect tumor cells in distant regions, whereas it can also cause secondary malignancy. As a result, our study can demonstrate that nonmetastatic local irradiation can also cause MN formation if large areas are given RT; thus, it may be necessary to avoid high dose rates to protect from secondary malignancy.

Ghandhi *et al*. have evaluated the long-term DNA damage in peripheral blood lymphocytes after whole thorax irradiation given in a high single dose in primates. They used the cytokinesis-block micronucleus assay to measure chromosomal aberrations as postmitotic micronuclei in blood samples collected up to 8 months after irradiation and found that significant induction of micronuclei in blood lymphocytes persisted with a gradual decline over the 8-month study period, indicating long-term DNA damage in blood lymphocytes after high single-dose irradiation.[23] Similarly, in the present study, we observed that RT given at a higher dose rate caused more cytogenetic damage. Therefore, we should be careful about the use of high dose rates in large field irradiation because of the abovementioned findings.

Cisplatin is used frequently to be radiosensitizer. It has cytotoxic effects to normal cells.[4,5] Cisplatin-induced DNA

damage may result in DNA fragmentation, chromosomal breaks, and MN formation causing genomic instability and may cause mutagenesis and carcinogenesis.[4] Similarly, in the present study, we found that RT-induced cytogenetic damage was further increased by cisplatin administration.

In conclusion, our findings suggest that RT with a higher dose rate causes more cytogenetic damage compared to RT with a standard dose rate, and this damage is increased by concurrent administration of cisplatin. More studies are needed for RT applications with high dose rates in cancer patients, in whom long-term survival is expected and the risk of a secondary malignity is high.

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## Conflicts of interest

There are no conflicts of interest.

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