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Modification of 10 cGy neutron or gamma-rays induced chromosomal damages by hyperthermia: an *in vitro* study

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Background: To evaluate the effects of hyperthermia (HT) on the frequency of chromosomal aberrations induced by a low dose of neutron or γ-rays in human peripheral blood lymphocytes. ***Materials and Methods:*** Blood samples were exposed to HT (41.5°C for 30 and 60min, 43°C for 15 and 30min), 10 cGy neutron or γ-rays, HT + neutron/γ, and neutron/γ + HT. After standard cell culture, harvesting, fixation and staining, the chromosomal damages were scored in metaphase plates. ***Results:*** HT alone at 41.5°C did not induce chromatid or chromosome aberrations, however, the frequency of damages was significantly higher at 43°C (P<0.05). Furthermore, the chromosomal damages was significantly different when cells were irradiated with neutron or γ-rays alone (P<0.01). HT 1 hr post neutron/γ irradiation significantly induced higher chromosome damages in comparison to HT 1 hr before irradiation (P<0.05). The chromosomal damages were remarkably higher when cells were irradiated with neutron then heated at 43°C for 30 min. ***Conclusion:*** Since increasing frequency of chromosome damages increases probability of cell death, application of HT after neutron irradiation (instead of X– or γ- rays) might be considered as a procedure for cells killing in radiotherapy. **Iran. J. Radiat. Res., 2009; 7 (2): 69-77**

Keywords: *Hyperthermia, neutron, gamma rays, chromosomal aberration, cancer.*

INTRODUCTION

One of the most important problems in radiotherapy (RT) with X and γ-rays is the presence of hypoxic cells in solid tumors. Due to insufficient blood perfusion, these cells are more resistant to RT. Survival of such cells at the end of RT may cause tumor recurrence. The oxygen enhancement ratio (OER) for X and γ-rays is 2.5-3. Thus, to reach the same cell damages in hypoxic conditions, one need to increase radiation dose to 2.5-3 times; that certainly will increase the absorbed dose in normal tissues around the tumor. During the recent decades the following attempts are considered to overcome the hypoxic cells: (a) hyperbaric oxygen, i.e. using O_2 with 2-3 atmosphere pressure during RT, (b) using drugs that specifically increase sensitivity of hypoxic cells, (c) applying high LET (linear energy transfer) radiation, *e.g.* neutron, and (d) hyperthermia (HT), i.e. the controlled increase of the tumor temperature by 3-8°C for 60-90 min [1].

Neutron has special characteristics which make it a unique radiation to overcome the hypoxic cells. For instance, it has high penetration capability, high LET [1], high RBE (relative biological effectiveness) [2], low OER [3], no dependence to the cell cycle, and finally, very low SLD (sub lethal damages) repair, and no PLD (potentially lethal damages) repair are performed after neutron irradiation [4]. However, there is still no worldwide application of neutron for RT. On the other hand, the hypoxic cells show a higher radiosensitivity in HT conditions [5, 6]. Therefore, combination of these two modalities may increase therapeutic gain in cancer treatment. Many researches have performed on the combination of HT and X or γ-rays but not so much for HT and neutron [7-9]. In the present study we used neutron and or γ irradiation in conjunction with HT.

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The purpose of this *in vitro* study was to investigate the effect of HT on the frequency of chromosomal aberrations induced by low dose of neutron or γ-rays. Previous studies demonstrated different results. While some researchers found that adding HT before irradiation decreased chromosomal damages [10, 11], other showed that using HT after irradiation increased the damages [12, 13]. In the present study, to compare the chromosomal aberrations, we applied HT 1 hr before and 1 hr after neutron and or γ irradiation.

MATERIALS AND METHODS

Samples

For each experiment 4-5 ml peripheral blood sample was taken from one person. The selected people were from non-smoking and non- previously irradiated 25-30 years old males. The study was approved by the Ethical Committee of the Tarbiat Modares University. All donors gave their informed written consent and completed a written questionnaire to obtain information related to their lifestyle, such as dietary habits, medical history and exposure to chemical and physical agents. Sterile and heparinized (5000 units per ml) syringe was used to take the blood from elbow vein. Blood was transferred to sterile flasks, under a laminar flow hood for various experiments.

Hyperthermia

For HT treatment a standard and calibrated incubator (Chemistry Technique, Tehran, Iran) was used. The culture flasks were simply placed in the incubator, similar to those used for cell culture. The incubator was kept "Turn On" to reach the specific temperature. The warming up took nearly 15 min which was excluded from HT duration time. Temperatures used were 41.5°C, for 30 and 60 min, and 43°C, for 15 and 30 min. Temperatures were controlled using a standard and calibrated thermometer with 5 -min intervals. Accuracy of the measurement was ±0.2°C with a precision of ±0.1°C. To end HT treatment, the samples were immediately moved to a 37°C incubator in which the specimens were kept for 30 min.

Neutron source

The neutron source was ^{252}Cf (Amersham, UK) available in Atomic Energy Organization, Tehran, Iran. The characteristics of the source were: half life: 2.645 years, dose rate: 1.52 $cGyhr^{-1}$, energy range: 1-6 MeV, medium energy: 2 MeV. The source was cylindrical in shape (diameter: 8 mm, height: 10 mm) which irradiated neutrons with an isotropic flux in 4π radian. Distance between centre of the sources and the centre of the sample containers was 3.5 cm.

Gamma source

The γ source was Co-60 (Theratron, AECL) available in Imam Hospital, Tehran, Iran. The characteristics of the source were: half life: 5.27 years, dose rate: 1.18 $Gymin^{-1}$, medium energy: 1.25 MeV. The source had a diameter of 20 mm. Distance between centre of the source and the centre of the sample containers was 80 cm. The neutron and γ doses used in this study were 10 cGy.

Experiments

***Control groups*:** One blood sample, as 1st control, was used for cell culture with no HT and no irradiation. One blood sample, as 2nd control, was kept in a 37°C incubator until other specimens were prepared for cell culture. The reason to select the 2nd control group was to evaluate the effect of environmental factors on the chromosomal damages during the time interval between two procedures and also carrying samples between main lab and neutron or γ labs. Samples were carried to the bab after irradiation on a flask filled with 37°C water.

***HT alone groups*:** Four samples were heated at 41.5°C, for 30 and 60 min, and at 43°C, for 15 and 30 min.

***Neutron alone irradiated group*:** One sample was irradiated by 10 cGy neutron alone.

***HT 1 hr before neutron*:** Two samples were firstly heated at 41.5°C, for 30 and 60 min; then 1 hr later they were irradiated with 10 cGy neutron. Between the two procedures, the samples were kept in a 37°C incubator.

***HT 1 hr after neutron*:** Two samples were firstly irradiated by 10 cGy neutron; then 1 hr later they were heated at 41.5°C, for 30 and 60 min. Between the two procedures, the samples were kept in a 37°C incubator. The last two experiments were performed also at 43°C, for 15 and 30 min.

In another part of the study the relation between the chromosome damages, induced by neutron and various HT duration time was evaluated. The selected heating duration times were 10, 20, 30, 40, 50, and 60 min; where HT was applied 1 hr after neutron irradiation.

Experiments with Gamma-rays: In similar conditions all the experiments performed with neutron irradiation, were repeated using 10 cGy γ-rays.
All experiments were repeated three times.

Cell culture and slide preparation

***Cell culture*:** To prepare cell culture, 0.4 ml of each blood sample (control, heated, irradiated, *etc.*) was added to 4 ml RPMI-1640 (Bahar Afshan, Iran) under a laminar flow hood supplemented with 15% fetal calf serum (Gibco), antibiotics (100 units / ml penicillin and 100 µg /ml streptomycin), 1% L-Glutamine, 0.1 ml phytohaemagglutinin (PHA) (supplied by Bahar Afshan, Iran), and 0.04 ml Bromodeoxyuridine (6.4 mg per 10 ml) (Sigma). The pH was kept around 7-7.4. Thereafter, the samples were kept in a 37°C incubator for 48 hrs. In the next step, 0.1 ml colchicines (0.02 mg per 100 ml) (Bahar Afshan) was added to each sample and the specimens were returned to the same incubator for 3 more hrs.

***Harvesting*:** The samples were centrifuged at 1000 rpm for 10 min and the supernatant was removed by suction. On a shaker 7 ml KCl (0.075 M) was added to the samples and left in a 37°C incubator for 20 min. The samples were then centrifuged (1000 rpm for 10 min) and the supernatant removed.

***Fixation*:** On a shaker 5 ml of a fresh fixative [3:1 methanol and glacial acetic acid (Merck)] was added to each sample drop wise. Then, the specimens were centrifuged (1000 rpm for 10 min) and the supernatant was removed. Cells were washed in fixative 2 more times.

***Slide preparation and staining*:** Using a Pasteur pipette, two drops of remaining fluid were thrown onto a clean and cold slide, previously stored in a freezer, from a 20-30 cm height. Two such slides were prepared for each sample. The slides were then placed in a gentle heat over a hotplate to dry. The cells were stained with Giemsa 5% (Merck) for 10 min. The slides were washed with distilled water and were dried at room temperature [14, 15].

Metaphase analysis

Using a light microscope (Ziess, Germany) with an 100× immersion oil objective, 100 well spread metaphase were scored for the presence of chromatid and chromosome type aberrations. Damages such as isochromatid breaks, deletions, sister unions, triradials, and quadriradials, were scored as chromatid type, while accentric fragments, dicentric, tricentric, and ring chromosomes were scored as chromosome type aberrations. Furthermore, the percentage of mitotic index (MI%) for each treatment was calculated using the following formula:

$$\mathrm{MI}\,\% = \frac{metaphases\ \ (in\,3000\ cells)}{3000} \times 100$$

All experiments were performed 3 times. Therefore, the numbers of evaluated metaphase cells were 300 in each group and the reported values are average per 100 metaphase cells.

Statistical analysis

Averages of chromosomal damages in two different groups were compared with Student's *t-test*. For evaluation of relation between chromosomal aberrations and HT duration time we used the regression test. The p-values are two-sided at a significance level of $p \leq 0.05$. SPSS software (version 12) was used for the statistical analysis.

RESULTS

Details of different chromatid type, chromosome type, and total aberrations found in this study are summarized in tables 1 and 2. In total, majority of the damages were of chromosome type, mainly chromosome exchange. No difference was found between chromatid, chromosome, or total aberrations of the 1st and the 2nd control groups. No difference was seen between chromatid, chromosome, or total damages of the samples heated at 41.5°C, neither for 30 nor for 60 min, and the control groups. No statistical difference was seen between chromatid type aberrations in samples heated at 43°C, neither for 15 nor for 30 min, and the control groups. A significant difference was found between chromosome type exchanges (and total aberrations) of the samples heated at 43°C, either for 15 or for 30 min, and the control groups ($p < 0.05$).

Mitotic indices

The range of calculated values for MI% was from 2.6 ± 0.18 to 4.2 ± 0.32 (tables 1 and 2). No difference was seen between MI% compared between two different groups (neither between two samples intra-group nor between two samples inter-groups).

Results of experiments with neutron irradiation

As summarized in table 1, a significant difference was seen between the frequency of chromatid, chromosome, and total aberrations of samples irradiated with 10 cGy neutron alone and the control groups ($p < 0.01$). however, no difference was found between chromatid, chromosome, or total aberrations for samples heated at 41.5°C, neither for 30 nor for 60 min, 1 hr before

Table 1. The mean and standard deviation (SD) of the frequency of chromatid and chromosome type aberrations induced by hyperthermia, 10 cGy of neutrons, and combination of hyperthermia with neutrons. The time interval between two procedures was 1 hour. 300 mitoses were analyzed and the data shown in the table are mean number per 100 cells. MI% shows percent of mitotic index.

Neutron/hyperthermia dose	Chromatid aberrations, ±SD			Chromosome aberrations, ±SD			Sum of damages, ±SD	MI%, ±SD
	Gaps	Deletions	Exchanges	Gaps	Deletions	Exchanges		
Control - I	0.2±0.2	0.25±0.2	0.3±0.3	0.5±0.4	0±0	0.5± 0.4	1.8±0.3	3.9± 0.11
Control - II	0±0	0± 0	0.5± 0.3	0±0	0.5±0.4	1±0.5	2.0± 0.2	3.1±0.14
41.5°C - 30min	0±0	0± 0	0.7± 0.5	0.25± 0.2	0.5± 0.4	1.25 ± 0.7	2.7± 0.3	2.6± 0.07
41.5°C - 60min	0.25±0.1	0.25± 0.1	0.7± 0.3	0.75±0.3	0.75± 0.2	2± 0.5	4.7± 0.3	3.3± 0.06
43.0°C - 15min	0.4±0.2	0.4± 0.3	1.3± 0.4	1.3± 0.4	1.7± 0.3	4±0.8	9.1±0.4	3.4± 0.21
43.0°C - 30min	0.7± 0.4	0.75± 0.4	1.3± 0.5	1.25± 0.5	0.75± 0.4	6.25±0.75	11± 0.5	3.5± 0.09
10 cGy Neutron	1± 0.3	0.5± 0.2	1.5± 0.5	2.25± 0.5	1.5± 0.4	8.25±0.6	15± 0.4	3.5± 0.06
41.5°C - 30min + 10cGy Neutron	0.5±0.2	1± 0.5	1.75± 0.8	2±0.7	1.5± 0.6	8.75± 0.8	15.5± 0.6	4.1± 0.05
41.5°C - 60min + 10cGy Neutron	0.75±0.5	0.75± 0.3	2.25± 0.4	2.25± 0.6	1.75± 0.5	8.5± 0.7	16.3± 0.5	3.1± 0.15
43.0°C - 15min + 10cGy Neutron	1.25± 0.4	1± 0.5	3± 0.5	2.75± 0.4	2.25± 0.4	10.8± 0.6	21.1± 0.5	3.9± 0.14
43.0°C - 30min + 10cGy Neutron	1.25± 0.5	1.5± 0.3	2.5± 0.6	3± 0.3	3± 0.7	11.25± 0.3	22.5± 0.5	3.5± 0.18
10 cGy Neutron + 41.5°C - 30min	2± 0.3	1.5± 0.8	2.2± 0.7	2± 0.5	1.75± 0.2	11.75±0.8	21.2± 0.6	3.9± 0.14
10 cGy Neutron + 41.5°C - 60min	1.5± 0.4	2± 0.6	2± 0.3	2.25± 0.5	2± 0.6	12.75± 0.4	22.5± 0.5	3.6± 0.18
10 cGy Neutron + 43.0°C - 15min	2± 0.6	1.75± 0.3	2.25± 0.5	3.25± 0.4	3± 0.2	14.25±0.4	26.5± 0.4	4.2± 0.25
10 cGy Neutron + 43.0°C - 30min	1.75±0.5	2.5± 0.4	2± 0.6	3.5± 0.3	3.5± 0.4	16.25± 0.3	29.5± 0.4	3.8±0.02

Table 2. The mean and standard deviation (SD) of the frequency of chromatid and chromosome type aberrations induced by hyperthermia, 10 cGy gamma rays, and combination of hyperthermia with gamma rays. The time interval between two procedures was 1 hour. 300 mitoses were analyzed and the data in the table are mean number per 100 cells. MI% shows percent of mitotic index.

Gammma/hyperthermia dose	Chromatid aberrations, ±SD			Chromosome aberrations, ±SD			Sum of damages, ±SD	MI%, ±SD
	Gaps	Deletions	Exchanges	Gaps	Deletions	Exchanges		
Control - I	0.2± 0.2	0.25±0.2	0.3±0.3	0± 0	0.2±0.1	0.2±0.2	1.2± 0.1	3.7± 0.09
Control - II	0±0	0± 0	0.5±0.3	0.1± 0.1	0.25± 0.2	0.6±0.4	1.5± 0.2	3.9± 0.12
41.5°C - 30min	0± 0	0± 0	0.7±0.5	0.1± 0.1	0.3± 0.2	0.75± 0.3	1.9±0.2	3.4±0.21
41.5°C - 60min	0.25± 0.1	0.25±0.1	0.7± 0.3	0.4± 0.2	0.4± 0.2	1± 0.4	3.0± 0.2	3.5± 0.08
43.0°C - 15min	0.4± 0.2	0.4± 0.3	1.3± 0.4	0.6±0.2	0.7± 0.3	2.25± 0.6	5.7± 0.3	3.6± 0.06
43.0°C - 30min	0.7± 0.4	0.75±0.4	1.3± 0.5	0.7±0.3	0.5±0.3	3.25± 0.5	7.2± 0.4	4.1±0.15
10 cGy Gamma	0.5± 0.2	0.3± 0.1	1± 0.5	1.25± 0.4	0.8±0.5	4± 0.6	7.9± 0.4	2.6± 0.18
41.5°C - 30min + 10cGy Gamma	0.3± 0.2	0.6± 0.2	1± 0.5	1± 0.3	0.9± 0.4	4.5± 0.7	8.3± 0.4	3.1± 0.05
41.5°C - 60min + 10cGy Gamma	0.4± 0.3	0.4± 0.1	1.25± 0.3	1.1± 0.4	1± 0.6	4.25± 0.8	8.4± 0.4	3.9±0.16
43.0°C - 15min + 10cGy Gamma	0.8± 0.2	0.45± 0.1	1.75± 0.6	1.5± 0.6	1.2±0.6	6.25± 0.6	12.0± 0.5	2.8± 0.09
43.0°C - 30min + 10cGy Gamma	0.5± 0.4	0.9± 0.3	1.4±0.4	1.75± 0.8	1.75±0.5	5.75± 0.5	12.1± 0.5	3.9±0.18
10 cGy Gamma + 41.5°C - 30min	1.1± 0.3	0.8±0.2	1.1± 0.5	1.1± 0.6	1.1± 0.2	6.25± 0.6	11.5± 0.4	4.2± 0.32
10 cGy Gamma + 41.5°C - 60min	0.8± 0.5	1±0.3	1.1±0.4	1.3± 0.4	1.2, ±0.4	6.5± 0.3	11.9± 0.4	3.8± 0.25
10 cGy Gamma + 43.0°C - 15min	1.2± 0.4	0.9±0.2	1.5± 0.6	2± 0.7	1.75± 0.6	7.25± 0.5	14.6± 0.5	3.6± .017
10 cGy Gamma + 43.0°C - 30min	1± 0.5	1.5± 0.5	1±0.5	1.8±0.5	2.25± 0.4	8.5± 0.6	16.1± 0.5	4.1± 0.08

neutron irradiation and those samples irradiated with neutron alone (table 1).

As shown in table 1, a significant difference was seen between chromatid exchanges for specimens heated at 43°C, either for 15 or for 30 min, 1 hr before neutron and those irradiated with neutron alone ($p<0.05$). Similarly, a significant difference was found between chromosome type aberrations for samples heated at 43°C, either for 15 or for 30 min, 1 hr before neutron and those irradiated with neutron alone ($p< 0.05$).

A significant difference was found between chromatid type aberrations of samples heated at 41.5°C, either for 30 or 60 min, 1 hr after neutron and those irradiated with neutron alone ($p<0.05$). A significant difference was also found between chromosome type exchanges of specimens heated at 41.5°C, either for 30 or 60 min, 1 hr after neutron and those irradiated with neutron alone ($p< 0.05$) (table 1).

A significant difference was also seen between chromatid type, chromosome type, or total aberrations for specimens heated at 43°C, either for 15 or for 30 min, 1 hr after neutron irradiation and those irradiated with neutron alone ($p< 0.01$).

Comparison of results after neutron and gamma irradiation

Comparing chromtid type, chromosome type, or total aberrations between two similar groups irradiated with neutron or γ-rays indicate that the frequency of the damages are roughly 1.2-2.0 times higher when cells were irradiated with neutron (compare tables 1 and 2).

Significantly higher frequency of chromosome type damages were seen in samples in which HT was applied 1 hr after irradiation (either with neutron or with γ-rays) in comparison to those HT was applied 1 hr before irradiation (compare tables 1 and 2). This was true for both temperatures (41.5°C for 30 and 60 min and 43°C for 15 and 30 min). P-values were different from <0.01 to <0.05. However, the maximum frequency of damages was found when cells were heated at 43°C for 30 min after exposure to10 cGy neutron irradiation.

Effect of heating duration time

Increasing duration time of HT (at 41.5°C) from 10 to 60 min, which applied 1 hr post 10 cGy of neutron or γ-irradiation, increased frequency of total chromosomal aberrations (figure 1). In both cases (neutron and γ-irradiation), strong correlations were seen between duration time of HT and the frequency of chromosomal aberrations. Nevertheless in total, the frequency of damages was higher when cells were irradiated with neutron in comparison to those samples irradiated with γ-rays.

DISCUSSION

This study demonstrates that applying HT 1 hr after 10 cGy of (neutron or γ-rays) causes significantly higher frequency of chromosomal type aberrations in human peripheral blood lymphocytes in comparison to cells that heated before irradiation. This study also shows that the frequency of chromosomal damages was significantly higher when cells were irradiated with neutron, in comparison to those irradiated with γ-rays.

During the recent decades there is an increased attention toward the use of combined modalities, *e.g.* RT+HT, or RT+chemotherapy for cancer treatment [16-20]. One of the most important rationales for using RT+HT is to overcome the problems of hypoxic cells, in the inner part of tumor. Hypoxic cells are relatively radioresistant, when RT is applied by X or γ-rays [21-23]. In comparison to X and γ-rays, neutron has various advantages for being used in radiotherapy (e.g., higher LET, higher RBE, and lower OER) [2, 4]. These characteristics may cause that neutron induces more biological effects, including chromosomal damages. Furthermore, when RT is applied by X or γ-rays, cells in the S-phase of the cell cycle show more resistance [1]. However, when neutron is used, there is no difference between radiosensitivity of the cells in the S -phase and cells in the other phases of the cell cycle [5]. In addition, studies show that when neutron is applied, a lower number of SLD repair and no PLD repair are seen in the damaged cells [24].

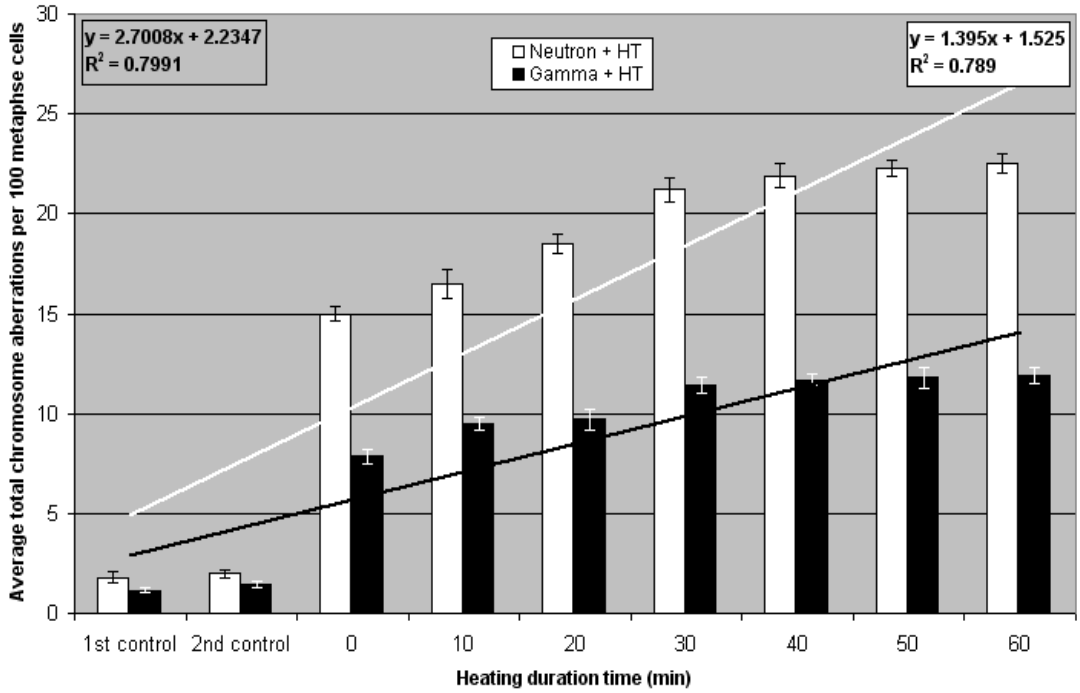

Figure 1. Average frequency of total chromosomal aberrations per 100 cells in human peripheral blood lymphocytes induced by 10 cGy neutron (or gamma) radiation and different duration time of hyperthermia (HT) at 41.5 °C. The time interval between irradiation and heating was 1 hour.

On the other hand, biological studies show that HT causes irreversible damage to the hypoxic cells, and that hypoxic cells are very sensitive to HT, especially in low pH conditions [7]. HT damages the membranes, cytoskeleton, and nucleus functions of the cells [25]. Temperatures above 41°C also pushes cancer cells toward acidosis (decreased cellular pH), which decreases the cells' viability and transplantability [26]. Moreover, tumor blood flow is increased by HT despite the fact that tumor-formed vessels do not expand in response to heat [27, 28]. Finally, heat preferentially affects on the cells in the S-phase of the cell cycle, which are known to be resistant to X or γ-rays, and make them more sensitive to RT [22-24].

The mentioned characteristics for neutrons and the mechanisms of HT may justify using additive complementary of neutron and HT for tumor cells killing, which of course needs further *in vivo* researches. Especially it might be a suitable procedure, when the neutron source could be implanted in the tumor or near the tumor.

In this study, applying HT alone, it was found a significant difference when cells were heated at 43°C, either for 15 or 30 min; however, no difference was found for mild HT (41.5°C), neither for 30 nor for 60 min. This finding is in agreement with the reports by Weissenborn and Obe [29, 30], who found no chromosomal damages in lymphocytes heated up to 41.5°C. These authors have discussed that temperatures between 37 and 41.5°C leading to reduced cellular damages and cell death [31-34].

In the present study we used a low dose (10 cGy) of neutron; however, we found significantly higher chromosome damages in the lymphocytes in comparison to non-irradiated cells. The results shown in tables 1 and 2, indicate that neutron by itself produce significant chromosomal damages in cells. These observation might be due to the DNA damages induced by low doses of neutrons as demonstrated by Maurizot *et al.* who found that low doses of fast neutrons could induce ssb (single strand break) and dsb (double strand break) in DNA of the plasmid of PBR322 [35].

Comparing chromosome damages in the cells irradiated with neutron alone and cells irradiated with neutron plus HT 1 hr later, it was found a higher number of damaged cells , demonstrating a higher effect of combined neutron and HT to induce chromosomal damages. Similarly, Szeinfeld *et al.* found higher number of cell damages in artificially hypoxic CaNT tumors, when HT was applied after neutron irradiation [36].

The highest frequency of chromosomal aberrations were seen when cells firstly irradiated with neutron; then heated at 43° C for 30 min, with a time interval of 1 hr. This finding is in agreement with the reports by Weissenborn and Obe who have seen higher cell damages when higher temperatures were used in combination with radiation [30, 31]. Applying HT 1 hr post neutron irradiation caused an increase in the chromosomal damages induced by neutron. This was true for both 41.5 and 43° C. Since HT itself at 41.5°C had no effect to induce the chromosomal damages, we may assume that HT increased the chromosomal damages by its prohibitory effect on the repair of the damaged cells. The following mechanisms might be involved in this process:

- HT affects on the activity of the enzymes. Spiro *et al.* showed that HT could inactivate the function of DNA-polymerase-ß enzyme in CHO cells which has important role in DNA repair [37]. Nevaldine also revealed that HT inhibits the repair of DNA damages induced by ionizing radiation [14]. Likewise, Raaphorst reported that HT inhibits the synthesis of DNA and RNA with inactivation of the enzymes function [38] and causes inactivation in DNA-polymerase α and ß enzymes in human glioblastoma, where this was more acceptable for DNA-polymerase-ß enzyme [39-42].
- Accumulation of non-histonic proteins.

Roti-Roti *et al.* showed that increasing of non-histonic proteins around a DNA molecule could inhibit the repair of the cell damages induced by irradiation [43-35].

- HT induces production of heat shock or stress proteins (HSPs). The induced HSPs could explain the effect of the inhibiting apoptotic signaling and acting as molecular chaperones to restrain irreversible cellular damage [46]. Molecular chaperones, which are mainly HSPs are involved in protein folding, refolding of partially denatured proteins, protein transport across membranes, cytoskeleton organization, and degradation of disabled proteins apoptosis. The HSPs also act as cytoprotective factors against deleterious environmental stresses [47].
- Inhibition of SLD and PLD repairs. Raaphorst showed that HT after irradiation prevents the repair of SLD and PLD and this process was directly related to temperature, HT duration time, and time interval between irradiation and HT [25].

In summary, HT by inactivation of enzymes, accumulation of proteins, and induction of HSPs, prevents the cells from repairing the damage sustained such as chromosomal aberrations. Since the cell death is directly related to the frequency of chromosomal type damages [39] this implies that using HT after neutron irradiation may cause more cell killing. Another finding in the present study was that increasing HT duration time increased the chromosomal damages induced by the low dose of neutron or γ-rays (figure 1). similar result was also reported by other investigators, showing direct relationship between heating duration time and cell damages induced by irradiation [30, 31, 48, 49].

The calculated mitotic indices, found in this study, were in the range of 2.6 to 4.2 (tables 1 and 2) and there was no different between MI% in two different groups. This result reveals that neither low dose of neutron (or γ-rays) nor HT, had cytotoxic effects on human lymphocytes. However, HT at 43°C, 10 cGy neutron (or γ-rays), and combinations of HT and neutron (or γ-rays) induced cytogenetic effect. The non cytotoxic effect of HT at 41.5 and 43°C is an advantage for this approach in cancer treatment modalities.

In conclusion the present study shows that the frequency of chromosomal aberrations was higher when hyperthermia was applied after neutron irradiation (instead of γ-rays). Since the frequency of damaged cells is directly related to the frequency of chromosomal aberrations, application of hyperthermia might improve the efficacy of radiotherapy with low doses of ionizing radiation.

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