



Chromosomal damage to human lymphocytes induced by hyperthermia pre and post extremely low dose neutron or gamma radiation

Daryoush Fatehi

Dept. of Medical Physics, Faculty of Medicine, Shahrekord University of Medical Sciences, Shahrekord, IRAN.

RESEARCH

Please cite this paper as: Fatehi D. Chromosomal damage to human lymphocytes induced by hyperthermia pre and post extremely low dose neutron or gamma radiation. AMJ 2010, 1, 4, 236-247 Doi 10.4066/AMJ.2010.138

Corresponding Author:

Dr. Daryoush Fatehi
Dept. of Medical Physics, Shahrekord
University of Medical Sciences,
Shahrekord, IRAN.
P.O. Box: 88155-571
Tel.: +98 (0381) 3335652
e-mail: d.fatehi@yahoo.com

Abstract

Background

One of the most important problems in radiotherapy (RT) with χ and γ -rays is hypoxic cells, in the centre of solids tumours. Due to insufficient blood perfusion, these cells are more resistant to RT. The purpose of the study is to assess the effect of heating cells on chromosomal damages induced by an extremely low dose of neutron or γ irradiation, in human lymphocytes.

Method

Human blood samples were exposed to hyperthermia (HT), 6 cGy neutron (or γ -rays), HT+neutron/ γ , and neutron/ γ +HT. HT was applied at 41.5°C for 30 and 60min as well as 43°C for 15 and 30min. The time interval between the two treatments was 1hr. After cell culture, harvesting, fixation, and staining, the chromosomal damages were scored in metaphase stage and statistical analyses were performed.

Results

In comparison to the control groups, HT alone at 41.5°C (neither for 30 nor 60min) did not induce significantly higher chromosomal damages ($p=0.8$); however, the number of damages was significantly higher at 43°C for 30min ($p<0.05$). Furthermore,

compared to the control groups the chromosomal damages was significantly different when cells irradiated with neutron/ γ -rays ($p<0.05$). Comparison between applying HT 1hr before and after irradiation, HT after neutron/ γ irradiation significantly induced higher chromosome damages ($p<0.05$). Comparing neutron and γ irradiation, the number of chromosomal damages was remarkably higher when cells irradiated with neutron ($p<0.01$).

Conclusion

Since applying an extremely low dose of neutron plus HT caused more chromosomal damages, in comparison to neutron/ γ alone, or HT plus neutron/ γ ; and because cell death is directly related to the chromosomal damage; thus, this combined regime might be considered as a treatment modality in cancer treatment.

Key Words

Hyperthermia, low dose, neutron, gamma, chromosomal aberration, cancer.

Background

One of the most important problems in radiotherapy (RT) with χ and γ -rays is hypoxic cells, in the centre of solids tumours. Due to insufficient blood perfusion, these cells are more resistant to RT [1]. Survival of such cells at the end of RT may cause tumour recurrence. The oxygen enhancement ratio (OER) for χ and γ -rays is 2.5-3. Thus, to reach the same cell damages in hypoxic conditions, one needs to increase radiation dose 2.5-3 times; that certainly will increase the absorbed dose in normal tissues around the tumour. During the last 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) rays, *e.g.* neutron, and (d) hyperthermia (HT), *i.e.* the controlled increased of the tumour temperature by 3-8°C for 60-90 min .



Neutron has special characteristics which make it a unique radiation to overcome the hypoxic cells. For instance, it has high power of penetration, high LET [ii], high RBE (relative biological effectiveness) [iii], low OER [iv], 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 [v]. However, there is still no a worldwide application of neutron for RT. On the other hand, the hypoxic cells have a higher radiosensitivity in HT conditions [vi, vii]. Therefore, combination of these two procedures may increase therapeutic gain in cancer treatment. Many studies have been performed with the combination of HT and α or γ -rays but not so much for HT and neutron [viii, ix]. In the present *in-vitro* study HT was used in conjunction with neutron and or γ irradiation to investigate the effect of HT on the frequency of chromosomal aberrations induced by low dose of neutron or γ . Previous studies demonstrated different results. While some researchers found that adding HT before irradiation decreased chromosomal damages [xi, xii], other showed that using HT after irradiation increased the damages [xiii, xiv]. Therefore, in this study we aimed to compare the chromosomal aberrations, by applying HT 1 hr before and 1 hr after neutron and or γ irradiation.

Method

Sample size:

For this study 12 people were selected randomly with simple sample drawing. The people were 25-30 year old male with no history of smoking and irradiation. The samples were chosen from volunteers who were going to donate some blood to the Iranian Blood Transfusion Organization, Tehran, Iran.

Blood samples:

For each experiment 45 cm³ peripheral blood specimen was taken from each sample person. Sterile and heparinized (5000 units per ml) syringe was used to take the blood sample from the elbow vein. The blood was transferred to sterile flasks, 3 ml per flasks, under a laminar flow hood. In this study human peripheral blood lymphocytes were used since they are very sensitive to ionizing radiation; therefore, a low dose of neutron or γ -rays can produce more chromosomal damages to these cells.

The following experiments were performed: Fifteen samples irradiated with 6 cGy neutron and HT: 1st control group, 2nd control group, HT at 41.5 °C (for 30 and 60 min), HT at 43 °C (for 15 and 30 min), 6 cGy neutron alone irradiation sample, HT at 41.5 °C for 30

min + 6 cGy neutron, HT at 41.5 °C for 60 min + 6 cGy neutron, HT at 43 °C for 15 min + 6 cGy neutron, HT at 43 °C for 15 min + 6 cGy neutron, 6 cGy neutron + HT at 41.5 °C for 30 min, 6 cGy neutron + HT at 41.5 °C for 60 min, 6 cGy neutron + HT at 43 °C for 15 min, and 6 cGy neutron + HT at 43 °C for 30 min. These fifteen experiments were repeated using γ -rays (see 1st column of tables 1 and 2).

The relation between the frequency of chromosome damage, induced by 6 cGy neutron irradiation, and HT duration time was evaluated performing the following nine experiments: 1st control group, 2nd control group, 6 cGy neutron alone irradiated samples, and 6 samples for which HT at 41.5 °C was added after 6 cGy neutron irradiation for 6 different duration times of 10, 20, 30, 40, 50 and 60 min. These nine experiments were also repeated using γ -rays.

For each group, the above 48 experiments were repeated three times in order to calculate the value of the average with more percussion. Therefore, in total 144 experiments were performed. The numbers of evaluated cells were 100 metaphase cells per group which means in total 14400 metaphase cells were evaluated to score the chromosomal damages.

Hyperthermia:

For HT a standard and calibrated incubator (Chemistry Technique, Tehran, Iran) was used. The 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 $\pm 0.2^\circ\text{C}$ with a precision of $\pm 0.1^\circ\text{C}$. To inhibit a heat shock, immediately after HT the samples were moved to a 37°C incubator in which the specimens were kept for 30 min.

Neutron source:

The Neutron source was ²⁵²/₉₈Cf (Amersham, UK) available in Iranian 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 4 π radian.



Gamma source:

The γ source was $^{60}_{27}\text{Co}$ available in Imam Hospital, Tehran, Iran. The characteristics of the source were: half life: 5.27 years, dose rate: 1.8 cGymin^{-1} , medium energy: 1.25 MeV. The source had diameter of 20 mm.

Distance between centre of the radiation source and the centre of the sample containers was 3.5 cm. The neutron and γ doses used in this study were 6 cGy.

Experiments set up:

The following experiments were performed on 144 blood samples to demonstrate the effect of HT on the frequency of chromosomal aberrations induced by a very low dose (6 cGy) of neutron (and or γ) rays in human peripheral blood lymphocytes.

Control groups: One blood sample, as 1st control, was used for cell culture with no HT and no irradiation. Another blood sample also with no HT and no irradiation, 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 the two procedures and also carrying samples between main lab and neutron or γ labs. The distance between the main lab and the other two labs was nearly 2 km. A flask filled with 37°C water was used to carry the samples.

HT alone groups: Four blood 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 blood sample was irradiated by 6 cGy neutron alone.

HT 1 hr before neutron irradiation: Two samples were firstly heated at 41.5 °C, for 30 and 60 min; then 1 hr later they were irradiated by 6 cGy neutron. Between the two procedures, the samples were kept in a 37°C incubator.

HT 1 hr after neutron irradiation: Two samples were firstly irradiated by 6 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 also repeated at 43 °C, for 15 and 30 min.

In another part of the study relation between the chromosome damages, induced by neutron irradiation, and HT duration time was evaluated. For this part of the study the volume of the taken blood sample was 27 cm^3 and the experiments were performed for the nine following groups: 1st and 2nd

control groups, 6cGy neutron irradiated group, and six groups for which HT (41.5 °C) was applied one hour after neutron irradiation. The selected heating duration times were 10, 20, 30, 40, 50, and 60 min.

Experiments with Gamma irradiation:

In similar conditions all the above 24 experiments performed with neutron irradiation, were repeated using 6 cGy γ -rays. All these 48 experiments were repeated three times.

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. Then, the following materials were added to the culture environment: 1 ml fetal calf serum (Gibco), 100 units per ml benzylpenicillin, 100 μg per ml streptomycin sulphate, 0.04 ml L-Glutamine, 0.1 ml phytohaemagglutinin, and 0.04 ml Bromodeoxyuridine (6.4 mg per 10 ml) (all by Bahar Afshan). 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 of the solution was removed by suction. On a shaker 7 ml KCl (0.075 mol per lit) was added to the samples and they were kept in the 37°C incubator for 20 min. Then, the samples were centrifuged (1000 rpm for 10 min) and the supernatant of the solution was removed.

Fixation: On a shaker 5 ml of a fresh solution was added [3:1 methanol and glacial acetic acid (both by Merck)] to each sample. Then, the specimens were centrifuged (1000 rpm for 10 min) and the supernatant of the solution was removed. This process was repeated 3 times.

Cell 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 placed in a gentle heat over a hotplate to dry. The cells were stained with Giemsa 5% (Merck) in which they were kept for 20 min. The slides were washed with distilled water and were dried in the lab temperature [^{xv}, ^{xvi}].

Analysis of metaphase:

Using a light microscope (Ziess, Germany) different chromatid and chromosome damages were scored in 100 metaphase cells. In this study, damages such as



isochromatid aberrations, sister unions, triradials, and quadriradials, were scored as chromatid exchanges, while accentric fragments, dicentric, tricentric, and ring chromosomes were scored as chromosome exchanges. Furthermore, the percent of mitotic index (MI%) was calculated using the following formula:

$$MI\% = \frac{\text{metaphases(in3000cells)}}{3000} \times 100$$

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

Statistical analysis: The ANOVA test was applied to compare the averages of chromosomal damages within the each procedure; *i.e.* neutron or γ irradiated groups, and Student T-test was used for comparing the results between the two procedures. To evaluate relation between chromosomal aberrations and HT duration time the regression test was used. The p-values are two-sided at a significance level of ≤ 0.05 . SPSS program (SPSS V 12, Chicago, ILL) was used for the statistical analysis.

Results

Details of different chromatid, chromosome, and total aberrations found in this study are summarized in tables 1 and 2. In total, majority of the damages were chromosomal type, mainly chromosome exchange. The following results were seen from comparison of the chromosomal damages in the different groups.

Control groups: No difference was found between chromatid, chromosome, or total aberrations of the 1st and the 2nd control groups ($p=0.9$).

HT alone at 41.5 °C: 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 ($p=0.8$).

HT alone at 43 °C: No difference was seen between chromatid damages of the samples heated at 43 °C, neither for 15 nor for 30 min, and the control groups ($p=0.9$). A significant difference was found between chromosome exchanges (and total damages) of the specimens heated at 43 °C, either for 15 or for 30 min, and the control groups ($p<0.05$).

Results of experiments with neutron irradiation

Neutron alone irradiated group: As shown in table 1 a significant difference was seen between the chromatid, chromosome, and total aberrations of samples irradiated with 6 cGy neutron alone and the control groups ($p<0.05$).

HT 1 hr before neutron irradiation: No difference was found between chromatid, chromosome, or total damages of the samples heated at 41.5 °C, neither for 30 nor for 60 min, 1 hr before neutron and those irradiated with neutron alone ($p=0.8$) (table 1).

Table 1 shows no difference was seen between chromatid exchanges of specimens heated at 43 °C, either for 15 or for 30 min, 1 hr before neutron and those irradiated with neutron alone ($p>0.1$). A significant difference was found between total damages of samples heated at 43 °C, either for 15 or for 30 min, 1 hr before neutron and those irradiated with neutron alone ($p<0.05$).

HT 1 hr after neutron irradiation: A significant difference was found between chromatid type damages of samples heated at 41.5 °C, either for 30 or 60 min, 1 hr after neutron and those irradiated with neutron alone ($p<0.05$). Also a significant difference was found between chromosome 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).

As table 1 reveals there is a significant difference between chromatid, chromosome, or total damages of specimens heated at 43 °C, either for 15 or 30 min, 1 hr after neutron irradiation and those irradiated with neutron alone ($p<0.05$).

Results of experiments with gamma irradiation

Gamma alone irradiated group: As table 2 shows a significant difference was seen between the chromatid, chromosome, and total aberrations of samples irradiated with 6cGy γ alone and the control groups ($p<0.05$).

HT 1 hr before gamma irradiation: No difference was found between chromatid, chromosome, or total damages of samples heated at 41.5 °C, neither for 30 nor for 60 min, 1 hr before γ and those irradiated with γ alone ($p=0.8$) (table 2).

As table 2 demonstrates a significant difference was seen between total damages of specimens heated at 43 °C, either for 15 or for 30 min, 1 hr before γ irradiation and those irradiated with γ alone ($p<0.05$).

HT 1 hr after gamma irradiation: No difference was found between chromatid gaps and deletions of samples heated at 41.5 °C, either for 30 or 60 min, 1 hr after γ and those irradiated with γ alone ($p>0.1$). However, a significant difference was found between chromosome exchanges of specimens heated at 41.5 °C, either for 30 or 60 min, 1 hr after γ and those irradiated with γ alone ($p<0.01$) (table 2).



As table 2 reveals a significant difference was seen between total damages of specimens heated at 43 °C, either for 15 or for 30 min, 1 hr after γ irradiation and those irradiated with γ alone ($p < 0.05$).

Comparison between neutron and gamma irradiation

Comparing chromatid, chromosome, or total aberrations between two similar groups irradiated with neutron or γ shows that frequency of the damages are roughly 1.2-2.0 times higher when cells irradiated with neutron ($p < 0.01$) (compare tables 1 and 2).

HT pre and post irradiation: Significantly higher frequency of chromosome type damages was seen in samples in which HT was applied 1 hr after irradiation (either with neutron or with γ -rays) in comparison to those in which HT was applied 1 hr before irradiation (compare tables 1 and 2) ($p < 0.01$ to < 0.05). This was observed for both temperatures (41.5 °C for 30 and 60 min, as well as 43 °C for 15 and 30 min). However, the maximum frequency of damages was found when cells heated at 43 °C for 30 min after 6 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 6 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 number of chromosomal aberrations. Nevertheless, in total the frequency of damages was higher when cells irradiated with neutron in comparison to those irradiated with γ -rays.

Mitotic Indices

The range of calculated values for MI% was from 2.6 ± 0.07 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) ($p > 0.8$).

Discussion

This study demonstrates that applying HT 1 hr after 6 cGy of (neutron or γ) causes significantly higher frequency of chromosomal type aberrations in human peripheral blood lymphocytes in comparison to cells that first heated then irradiated. This study also shows that the frequency of chromosomal damages was significantly higher when cells irradiated with a very low dose of neutron, in comparison to those irradiated with γ -rays.

During the last decades there is an increasing attention to use combined regimens, *e.g.* RT+HT, or RT+ chemotherapy for cancer treatment [^{xvii}, ^{xviii}, ^{xix}, ^{xx}, ^{xxi}]. One of the most important rationales for using RT+HT is to overcome the hypoxic cell, in the inner part of tumour. Hypoxic cells are relatively radioresistant, when RT is applied by χ or γ -rays [^{xxii}, ^{xxiii}, ^{xxiv}]. In comparison to χ and γ -rays, neutron has a higher LET, a higher RBE, and a lower OER [ii, iii, iv]. These characteristics may cause neutron to induce more biological effects, including chromosomal damages. Especially in the hypoxic cells, neutron could cause more cell damages; since it has less dependence to oxygen (OER for neutron is 1.6 vs. 2.5-3 for χ and γ -rays).

Furthermore, when RT is applied by χ or γ -rays, cells in the S-phase of the cell cycle show more resistance [i]. 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 [v]. Additionally, studies show that when neutron is applied, a lower number of SLD repair and no PLD repair is seen in the damaged cells [^{xxv}]. On the other hand, studies on biological aspect show that HT causes irreversible damage to the hypoxic cells, and that hypoxic cells are very sensitive to HT, especially in low pH conditions [vii]. HT damages the membranes, cytoskeleton, and nucleus functions of the cells [^{xxvi}]. Temperatures above 41°C also push cancer cells toward acidosis (decreased cellular pH), which decreases the cells' viability and transplantability [^{xxvii}]. Moreover, tumour blood flow is increased by HT despite the fact that tumour-formed vessels do not expand in response to heat [^{xxviii}, ^{xxix}]. Finally, heat preferentially affects on the cells in the S-phase of the cell cycle, which are known to be resistant to χ or γ -rays, and make them more sensitive to RT.

The above-mentioned characteristics of neutron and the mechanisms of HT may justify using additive complementary of neutron and HT for tumour 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 tumour or near the tumour.

In this study, when HT was applied alone, significant difference was reached 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. The similar findings were also reported by Weissenborn and Obe [^{xxx}, ^{xxxi}], where they found no chromosomal damages in lymphocytes heated up to



41.5 °C. They concluded that temperatures between 37 and 41.5 °C might increase thermotolerance which inhibits increasing of the chromosomal damages and cell death [xxxii, xxxiii, xxxiv, xxxv].

Although a very low dose (6 cGy) of neutron was used in the present study; significantly higher chromosome damages were found in the human lymphocytes in comparison to non irradiated cells. One may believe that the effect of low doses of neutrons on cell damages is due to γ component of the neutron source ($^{252}_{98}\text{Cf}$). However, the results of this study, by comparing frequency of the damages between tables 1 and 2, show that neutron itself is mainly responsible for the cell damages. Likewise, Maurizot *et al.* 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 [xxxvi].

Comparing chromosome damages in the cells irradiated with neutron alone and cells irradiated with neutron plus HT 1 hr later, we found a higher number of cells damages, demonstrating a higher effect of combined neutron and HT to induce chromosomal damages. Szeinfeld *et al.* also found higher number of cell damages in CaNT tumours, which were artificially hypoxic, when HT used after neutron irradiation [xxxvii].

In the present study the highest chromosomal damages was seen when cells firstly irradiated with neutron; then heated at 43 °C for 30 min, with a time interval of 1 hr. This is in agreement with Weissenborn and Obe who have seen higher cell damages when higher temperatures were used in combination with radiation.

The present study showed that applying HT 1 hr post neutron irradiation caused an increase in the chromosomal damages induced by a very low dose of neutron irradiation. This phenomenon was noticed for both 41.5 and 43 °C. Since HT itself at 41.5 °C had no effect on inducing the chromosomal damages, we may assume that HT increased the chromosomal damages by its prohibitory effect on the repair of the damaged cells.

HT by inactivation of enzymes, accumulation of proteins, and induction of HSPs, prevents the cells from repairing the damage sustained such as chromosomal aberrations [xxxviii, xxxix, xl]. Since the cell death is directly related to the frequency of chromosomal type damages [xli] this implies that using HT after neutron irradiation may causes more cell killing.

Another finding in this study was that increasing HT duration time increased the chromosomal damages induced by the very low dose of neutron or γ . This result was also found by others, showing direct relation between heating duration time and cell damages induce by irradiation [xlii, xliii].

The calculated MI%, found in this study, are in a range of 2.6 to 4.2 (tables 1 and 2, last column) and there was no difference between MI% for two different groups. This result reveals that neither a very low dose of neutron (or γ -rays) nor HT, have cytotoxic effect on human lymphocytes. However, HT at 43 °C, 6 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 the present study comparing neutron and gamma irradiation, we found that frequency of total chromosomal aberrations was roughly doubled when cells firstly irradiated with neutron then heated at 41.5 or 43 °C. This result shows that one may consider using the combination of "neutron plus HT" as a replacement for conventional cancer treatment. However, in RT tumour cells are the main target for treatment, but in the present study only the normal cells were exposed to irradiation. This is one of the study limitations. The point is that if neutron plus HT can induce more damages in normal cells, will the enhancement be the same in the tumour cells. Thus, further research is needed to perform more experiments on tumour cells exposed with neutron and HT.

Conclusion

In comparison to neutron or gamma irradiation alone, as well as hyperthermia before neutron or gamma irradiation, applying hyperthermia after a very low dose of neutron, increases the frequency of chromosomal damages in human lymphocytes. Since cell death is directly related to the frequency of the cell damages; using hyperthermia after neutron irradiation might be considered as an effective procedure for tumour cell killing in radiotherapy.



References

- ¹. Hall EJ, Giaccia AJ. (2005) Radiobiology for Radiologist. 6th ed., Philadelphia, JB Lippincott Williams & Wilkins.
- ⁱⁱ Bewley DK. (1971) Physical characteristics of fast neutron beams. *Eur J Cancer* 7(2):99-104.
- ⁱⁱⁱ. Broerse JJ, Barendsen GW. (1973) Relative biological effectiveness of fast neutrons for effects on normal tissues. *Curr Topics, Radiat Res Q* 8(4):305-350.
- ^{iv}. Broerse JJ, Barendsen GW, van Kersen GR. (1968) Survival of cultured human cells after irradiation with fast neutrons of different energies in hypoxic and oxygenated conditions. *Int J Radiat Biol Relat Stud Phys Chem Med* 13(6):559-572.
- ^v. Schneider DO, Whitmore GF. (1963) Comparative effects of neutrons and x-rays on mammalian cells. *Radiat Res* 18:286-306.
- ^{vi}. Hall EJ, Roizin-Towle L. (1984) Biological effects of heat. *Cancer Res* 44(10 Suppl):4708s-4713s.
- ^{vii}. Overgaard J, Grau C, Lindegaard JC, Horsman MR. (1991) The potential of using hyperthermia to eliminate radioresistant hypoxic cells. *Radiother Oncol* 20 (11Supp):113-116.
- ^{viii}. Fatehi D. (1996) The genetic effect of neutrons on cells treated with hyperthermia. MSc-thesis.
- ^{ix}. Fatehi D. (1999) Effect of hyperthermia before neutron irradiation on chromosomal damages of human lymphocytes. *Shahrekord University of Medical Sciences' Journal* 1(3):55-60.
- ^x. Fatehi D, Mozdarani H. (2002) Effect of hyperthermia after neutron irradiation on chromosomal aberration frequency in human peripheral blood lymphocytes. *Shahrekord University of Medical Sciences' Journal* 4(1):55-61.
- ^{xi}. Cai L, Jiang M. (1995) Mild hyperthermia can induce adaptation to cytogenetic damage caused by subsequent X-irradiation. *Radiat Res* 143:26-33.
- ^{xii}. Nielsen OS. (1983) Influence of thermotolerance on the interaction between hyperthermia and radiation in L1A2 cells in vitro. *Int J Radiat Biol Relat Stud Phys Chem Med* 43(6):665-673.
- ^{xiii}. Mittler S. (1984) Hyperthermia increases chromosome breakage and loss induced by fission neutrons in *Drosophila melanogaster*. *Mutat Res* 139(3):119-121.
- ^{xiv}. Nevaldine B, Longo JA, Hahn PJ. (1994) Hyperthermia inhibits the repair of DNA double-strand breaks induced by ionizing radiation as determined by pulsed-field gel electrophoresis. *Int J Hyperthermia* 10(3):381-388.
- ^{xv}. Evans HJ, O'Riordan ML. (1975) Human peripheral blood lymphocytes for the analysis of chromosome aberrations in mutagen tests. *Mutat Res* 31(3):135-148.
- ^{xvi}. International Atomic Energy Agency. (1986) Biological Dosimetry: Chromosomal aberration analysis for dose assessment. Technical report series. Vienna, Austria. 260:59-63.
- ^{xvii}. Kim TH, Kim DY, Cho KH, Kim YH, Jung KH, Ahn JB, Chang HJ, Kim JY, Choi HS, Lim SB, Sohn DK, Jeong SY. (2005) Comparative analysis of the effects of belly board and bladder distension in postoperative radiotherapy of rectal cancer patients. *Strahlenther Onkol* 181:601-605.
- ^{xviii}. Dunst J, Diestelhorst A, Kuhn R, Muller AC, Scholz HJ, Fornara P. (2005) Organ-sparing treatment in muscle-invasive bladder cancer. *Strahlenther Onkol* 181:632-637.
- ^{xix}. Windschall A, Ott OJ, Sauer R, Strnad V. (2005) Radiation therapy and simultaneous chemotherapy for recurrent cervical carcinoma. *Strahlenther Onkol* 181:545-550.
- ^{xx}. Hildebrandt B, Wust P. (2007) The biologic rationale of hyperthermia. *Cancer Treat Res* 134:171-184.
- ^{xxi}. Hildebrandt B, Wust P. (2007) Interaction between hyperthermia and cytotoxic drugs. *Cancer Treat Res* 134:185-193.
- ^{xxii}. Gerweck K, Gillette EL, Dewey WC. (1974) Killing of Chinese hamster cells in vitro by heating under hypoxic or anaerobic conditions. *Am J Cancer* 10:691-693.
- ^{xxiii}. Kim SH, Kim JH, Hahn EW. (1975) Enhanced killing of hypoxic tumour cells by hyperthermia. *Br J Radiol* 48:872-874.
- ^{xxiv}. Freeman ML, Dewey WC, Hopwood LE. (1977) Effect of pH on hyperthermic cell survival: brief communication. *J Natl Cancer Inst* 58:1837-1839.
- ^{xxv}. Raaphorst GP. (1992). Recovery of sublethal radiation damage and its inhibition by hyperthermia in normal and transformed mouse cells. *Int J Radiat Oncol Biol Phys* 22(5):1035-1041.
- ^{xxvi}. Song CWM, Shakil A, Griffin RJ, Okajima K. (1997) Improvement of tumour oxygenation status by mild temperature hyperthermia alone or in combination with carbogen. *Semin Oncol* 24:626-632.
- ^{xxvii}. Endrich B, Zweifach BW, Reinhold HS, Intaglietta M. (1979) Quantitative studies of microcirculatory function in malignant tissue. *Int J Radiat Oncol Biol Phys* 5:2021-2030.
- ^{xxviii}. Song CWM, Rhee JG, Levitt SH. (1980) Blood flow in normal tissues and tumours during hyperthermia. *J Natl Cancer Inst* 64:119-124.
- ^{xxix}. Kang MS, Song CW, Levitt SH. (1980) Role of vascular function in response of tumours in vivo to hyperthermia. *Cancer Res* 40:1130-1135.
- ^{xxx}. Weissenborn U, Obe G. (1991) Modification of X-ray induced chromosome aberration frequency by pre- and postirradiation hyperthermia of human peripheral lymphocytes. *Int J Radiat Biol* 59(4):973-984.



- ^{xxxix}. Weissenborn U, Obe G. (1992) Modification of bleomycin-induced chromosome aberrations by hyperthermia and under energy depleting conditions in human peripheral lymphocytes. *Int J Radiat Biol* 62(3):289-296.
- ^{xxxix}. Raaphorst GP, Mao J, Ng CE. (1995) Thermotolerance in human glioma cells. *Int J Hyperthermia* 11(4):523-529.
- ^{xxxix}. Raaphorst GP, Mao JP, Ng CE. (1998) Thermotolerance effects on thermoradiosensitization in human glioma cells. *Int J Hyperthermia* 14(1):85-95.
- ^{xxxix}. Nielsen OS. (1983) Influence of thermotolerance on the interaction between hyperthermia and radiation in L1A2 cells in-vitro. *Int J Radiat Biol Relate Stud Phys Chem Med* 43(6):665-673.
- ^{xxxix}. Dewhirst MW, Vujaskovic Z, Jones E, (2005) Thrall D. Re-setting the biologic rationale for thermal therapy. *Int J Hyperthermia* 21(8):779-790.
- ^{xxxix}. Maurizot SM, Charlier M, Sabatier R. (1990) DNA radiolysis by fast neutrons. *Int J Radiat Biol* 57(2):303-313.
- ^{xxxix}. Szeinfeld D, de Villiers N, Wynchank S. (1992) Modification of the effects of hyperthermia and neutron radiation on the activity of acid phosphatase in CaNT tumours. *Cancer Biochem Biophys* 12(4):253-261.
- ^{xxxix}. Raaphorst GP, Yang DP, Bussey A, Ng CE. (1995) Cell killing, DNA polymerase inactivation and radiosensitization to low dose rate irradiation by mild hyperthermia in four human cell lines. *Int J Hyperthermia* 11(6):841-854.
- ^{xxxix}. Roti Roti JL, Henle KJ, Winward RT. (1979) The kinetics of increase in chromatin protein content in heated cells: a possible role in cell killing. *Radiat Res* 78(3):522-531.
- ^{xxxix}. Coss RA. (2005) Inhibiting induction of heat shock proteins as a strategy to enhance cancer therapy. *Int J Hyperthermia* 21(8):695-701.
- ^{xxxix}. Raaphorst GP. (1990) Fundamental aspects of hyperthermic biology. In: Field SB, Hand JW, Jeffrey W, editors. *An introduction to practical aspects of clinical hyperthermia*. 1st ed. London, UK Taylor and Francis. pp 90-98.
- ^{xxxix}. Mitchel RE, Birnboim HC. (1985) Triggering of DNA strand breaks by 45 degrees C hyperthermia and its influence on the repair of gamma-radiation damage in human white blood cells. *Cancer Res* 45(5):2040-2045.
- ^{xxxix}. Hume SP, Myers R, Field SB. (1982) A comparison of thermal enhancement ratios for fast neutron and x irradiation of two normal tissues in rodents. *Br J Radiol* 55(650):151-155.



Figures and Tables

Table 1. The average and standard deviation (SD) of frequency of chromatid and chromosome aberrations induced by hyperthermia, 6 cGy of neutron, and combination of hyperthermia and neutron. The time interval between two treatments was 1 hour. Numbers of evaluated metaphase cells were 300 and the numbers in the table are averages per 100 cells. Damages such as isochromatid aberrations, sister unions, triradials, and quadriradials, were scored as chromatid exchanges, while acacentric fragments, dicentric, tracentric, and ring chromosomes were considered as chromosome exchanges. MI% shows percent of mitotic index (see text how it was calculated).

Treatment (Neutron/hyperthermia dose)	Chromatid aberrations, 1SD			Chromosome aberrations, 1SD			Sum of damages, 1SD	MI%, 1SD
	Gaps	Deletions	Exchanges	Gaps	Deletions	Exchanges		
Control – I	0.1, 0.1	0.15, 0.1	0.1, 0.1	0.2, 0.2	0.1, 0.1	0.3, 0.2	0.95, 0.1	2.6, 0.07
Control – II	0.1, 0.1	0.1, 0.1	0.3, 0.2	0, 0	0.25, 0.1	0.5, 0.2	1.25, 0.1	3.4, 0.21
41.5 °C – 30 min	0.1, 0.1	0.1, 0.1	0.2, 0.3	0.1, 0.1	0.25, 0.2	0.7, 0.3	1.45, 0.2	3.9, 0.11
41.5 °C – 60 min	0.15, 0.1	0.15, 0.1	0.4, 0.3	0.5, 0.2	0.5, 0.2	1, 0.5	2.7, 0.2	3.5, 0.08
43.0 °C – 15 min	0.3, 0.1	0.3, 0.2	0.8, 0.4	0.8, 0.3	1.1, 0.3	1.2, 0.8	4.5, 0.4	3.1, 0.14
43.0 °C – 30 min	0.5, 0.2	0.35, 0.3	0.9, 0.35	0.95, 0.4	0.5, 0.2	1.5, 0.5	4.7, 0.3	3.3, 0.06
6 cGy Neutron	0.7, 0.3	0.3, 0.15	1, 0.5	1.35, 0.3	1.5, 0.3	5.15, 0.6	10, 0.4	3.9, 0.14
41.5 °C – 30min + 6 cGy Neutron	0.5, 0.2	0.6, 0.3	1.2, 0.6	1.4, 0.5	1.6, 0.4	5.2, 0.8	10.5, 0.5	3.9, 0.14
41.5 °C – 60 min + 6 cGy Neutron	0.6, 0.3	0.7, 0.3	1.3, 0.4	1.5, 0.5	1.4, 0.5	5.7, 0.6	11.2, 0.4	3.5, 0.18
43.0 °C – 15 min + 6 cGy Neutron	0.8, 0.4	0.8, 0.5	1.8, 0.5	1.7, 0.3	1.3, 0.4	5.8, 0.7	12.2, 0.5	4.1, 0.05
43.0 °C – 30 min + 6 cGy Neutron	0.9, 0.5	1, 0.3	1.5, 0.4	1.4, 0.7	1.4, 0.6	6.3, 0.4	12.5, 0.5	3.1, 0.15
6 cGy Neutron + 41.5 °C – 30 min	1.2, 0.2	1.1, 0.4	1.5, 0.5	1.4, 0.6	1.5, 0.5	6.5, 0.5	13.2, 0.5	4.2, 0.25
6 cGy Neutron + 41.5 °C – 60 min	1.3, 0.3	1.2, 0.4	1.6, 0.3	1.5, 0.5	1.5, 0.6	6.8, 0.4	13.9, 0.4	3.8, 0.02
6 cGy Neutron + 43.0 °C – 15 min	1.5, 0.4	1.5, 0.25	1.75, 0.4	2.3, 0.6	2.2, 0.7	7.25, 0.3	16.5, 0.4	3.5, 0.06
6 cGy Neutron + 43.0°C - 30min	1.75, 0.5	2, 0.3	1.8, 0.4	2.4, 0.4	2.5, 0.5	8.25, 0.5	18.7, 0.4	3.6, 0.18

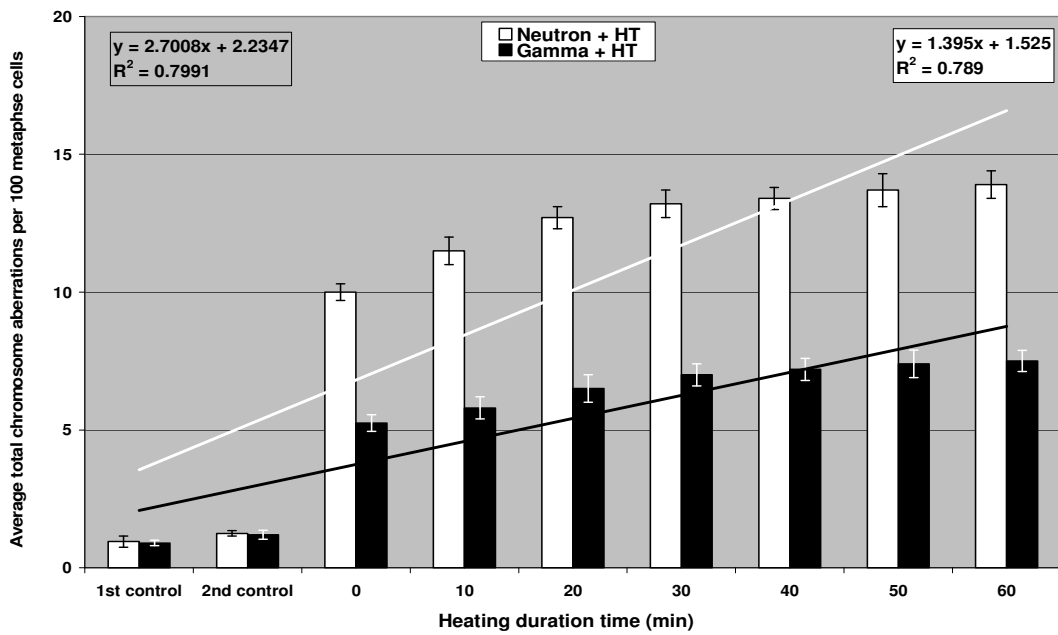


Table 2. The average and standard deviation (SD) of frequency of chromatid and chromosome aberrations induced by hyperthermia, 6 cGy of gamma, and combination of hyperthermia and gamma. The time interval between two treatments was 1 hour. Numbers of evaluated metaphase cells were 300 and the numbers in the table are averages per 100 cells. Damages such as isochromatid aberrations, sister unions, triradials, and quadriradials, were scored as chromatid exchanges, while acentric fragments, dicentric, tracentric, and ring chromosomes were considered as chromosome exchanges. MI% shows percent of mitotic index (see text how it was calculated).

Treatment (Gamma/hyperthermia dose)	Chromatid aberrations, 1SD			Chromosome aberrations, 1SD			Sum of damages, 1SD	MI%, 1SD
	Gaps	Deletions	Exchanges	Gaps	Deletions	Exchanges		
Control – I	0.1, 0.1	0.1, 0.1	0.1, 0.1	0.2, 0.1	0.2, 0.1	0.2,0.2	0.9, 0.1	3.1, 0.05
Control – II	0.2, 0.1	0.1, 0.1	0.2, 0.2	0.1, 0.1	0.2, 0.2	0.4,0.3	1.2, 0.2	4.1, 0.15
41.5 °C – 30 min	0.1, 0.1	0.2, 0.1	0.2, 0.1	0.1, 0.1	0.3, 0.2	0.5, 0.2	1.4, 0.1	3.6, .017
41.5 °C – 60 min	0.15 0.2, 0.4,	0.2, 0.15	0.6, 0.4	0.5, 0.2	0.5, 0.25	0.9, 0.3	2.9, 0.2	3.9, 0.16
43.0 °C – 15 min	0.3	0.4, 0.2	1, 0.5	0.6, 0.3	0.7, 0.4	1.3, 0.5	4.4, 0.4	4.2, 0.32
43.0 °C – 30 min	0.3, 0.2	0.5, 0.3	1, 0.4	0.9, 0.3	0.7, 0.3	1.2, 0.6	4.6, 0.4	3.4, 0.21
6 cGy Gamma	0.4, 0.3	0.5, 0.2	0.9, 0.4	1, 0.3	0.8, 0.4	1.65, 0.4	5.25, 0.3	3.8, 0.25
41.5 °C – 30 min + 6 cGy Gamma	0.3, 0.2	0.6, 0.3	1, 0.3	1, 0.4	0.85, 0.3	1.7, 0.5	5.45, 0.3	2.8, 0.09
41.5 °C – 60 min + 6 cGy Gamma	0.4, 0.3 0.45,	0.4, 0.1	1.25, 0.3	1.1, 0.4	1, 0.6	1.85, 0.8	6, 0.4	4.1, 0.08
43.0 °C – 15 min + 6 cGy Gamma	0.1	0.6, 0.2	1.05, 0.3	1.1, 0.5	1.1, 0.4	2, 0.6	6.3, 0.4	3.5, 0.08
43.0 °C – 30 min + 6 cGy Gamma	0.4, 0.2	0.4, 0.3	1, 0.4	1.2, 0.8	1.25, 0.5	2.25, 0.5	6.5, 0.5	3.9, 0.18
6 cGy Gamma + 41.5 °C – 30 min	0.6, 0.4	0.5, 0.2	0.9, 0.3	1.1, 0.7	1.1, 0.2	2.8, 0.4	7, 0.4	3.1, 0.05
6 cGy Gamma + 41.5 °C – 60 min	0.5, 0.3	0.7, 0.4	1., 0.5	0.9, 0.3	1, 0.6	3.4, 0.7	7.5, 0.5	2.6, 0.18
6 cGy Gamma + 43.0 °C – 15 min	0.7, 0.2	0.9, 0.3	1.1, 0.5	1, 0.4	1.05, 0.5	3.5, 0.8	8.25, 0.5	3.6, 0.06
6 cGy Gamma + 43.0 °C – 30 min	0.9, 0.4	1.1, 0.5	1.1, 0.4	1.2, 0.6	1.25, 0.3	3.95, 0.4	9.5, 0.4	3.9, 0.12



Figure 1. Average frequency of total chromosome aberrations per 100 cells in human peripheral blood lymphocytes induced by 6 cGy neutron (or gamma) irradiation and different duration time of hyperthermia (HT) at 41.5 °C. The time interval between irradiation and heating was 1 hour.





PEER REVIEW

Not commissioned. Externally peer reviewed.

CONFLICTS OF INTEREST

The authors declare that they have no competing interests.

