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Estimation of relative biological effectiveness of 6 and 22 MeV neutrons and ^{137}Cs γ -rays based on the analysis of chromosome aberrations in human peripheral blood lymphocytes

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Summary

Aim	This investigation presents the estimation of cytogenetic injury in human peripheral blood lymphocytes and its dependence on the dose and quality of ionizing radiation applied in radiotherapy of cancer patients with the purpose of calculating RBE values for different radiation sources in the dose range under study.
Materials/Methods	Cytogenetic dosimetry was carried out at an exposure of human lymphocyte cultures to ^{137}Cs and ^{60}Co therapeutic sources and neutrons with average energies of 6 and 22 MeV.
Results	Increase in dose reduces RBE values of ^{137}Cs γ -irradiation, which in the range of doses investigated (0.3–5.0 Gy) vary between 3.5–1.3, respectively. Thus the difference in RBE values can be most accurately determined in the 0.5–2.0 Gy dose interval usually used in radiotherapy. Comparative estimation of fast neutron RBE values showed that 6 MeV neutrons give significantly higher yields of chromosome aberrations as compared with 22 MeV neutrons. It was shown that cytogenetic effectiveness has a tendency to decrease with the increase in the depth of the water phantom. The largest effect was observed at the depth of up to 6 cm.
Conclusions	The results of our pre-clinical studies indicate that radiation-induced effects simultaneously depend on different irradiation parameters such as dose, energy and the depth of the irradiated biological object, which should be taken into account in radiotherapy of cancer patients.
Key words	^{137}Cs γ -photons • neutrons • chromosome aberrations • relative biological effectiveness • radiotherapy

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BACKGROUND

The examination of radiobiological parameters of ionizing radiation from radiotherapeutic sources afford opportunity to choose the necessary exposure requirements for patients. A therapeutic ionising radiation source should be examined radiobiologically including the evaluation of its relative biological effectiveness (RBE) dependent on radiation quality, dose, cell radiosensitivity etc. [1]. The investigation of radiation-induced cytogenetic injury levels in human cells seems to be a good method of investigation due to the leading role of structural chromosome rearrangements in the development of radiation damage and post-radiation cell death [2].

It is known that RBE estimation of a new ionising radiation is the most important piece of information in clinical radiobiology and radiotherapy. The exact knowledge of RBE values is necessary for correct planning of radiation burden on normal and tumour tissues. The absence of convincing literature data on the biological effectiveness of ^{137}Cs γ -radiation required that its RBE be estimated for intracavitary radiotherapy of cancer patients at the Institute of Oncology of AMS of Ukraine, based on the estimation of chromosome changes in human peripheral blood lymphocytes and on its comparison with the effects of a traditional ^{60}Co γ -source. Application of 6 MeV neutrons in radiotherapy of human tumours in Ukraine, based on the Bio-medical Complex of U-120 Cyclotron at the Institute of Nuclear Research of NAS of Ukraine, gave us the opportunity to carry out additional radiobiological research of neutron RBE dependence on various parameters of irradiation.

AIM

This investigation presents data on the estimation of cytogenetic damage of human peripheral blood lymphocytes and its dependence on the dose and quality of ionising radiation applied in radiotherapy of cancer patients with the purpose of calculating RBE values for various radiation sources in the dose range investigated.

MATERIALS AND METHODS

Biological dosimetry was carried out at an exposure of human lymphocyte cultures to therapeutic sources of ^{137}Cs ("Selectron"), ^{60}Co ("Rocus"), neutrons with average energy of 6 and 22 MeV (U-120 and U-240 Cyclotron). The test system of human peripheral blood lymphocyte culture with metaphase analysis of chromosomal aberrations was used as a method of biological dosimetry [2]. Lymphocyte cultures were established from blood samples of 20 patients according to the modified method of Moorhead [3]. After exposure, lymphocytes were incubated in a RPMI-1640 medium containing 0.2 $\mu\text{g}/\text{ml}$ PHA (Difco, USA) and 15% foetal calf serum for 52 hours (2 last hours with colchicine). This procedure made it possible to analyse cells in the first post-radiation mitosis. After hypotonic treatment with 75 mM KCl, lymphocytes were treated with freshly prepared 3:1 methanol/acetic acid fixative. Then the cell suspension was applied to frost wet slides and dried. Preparations were hydrolysed in 5N HCl, washed and stained with 2% Giemsa solution.

The analysis of stained chromosome preparations was accompanied by visual karyotyping. Chromatid-type (interchang-

ings, deletions, isodeletions) and chromosome-type (paired fragments, minute chromosomes, rings, dicentric and anomalous monocentric) aberrations were scored. 300–400 metaphases were analysed for each point examined.

Lymphocyte cultures were exposed to fast neutrons with an average energy of 6 MeV in the 0.05–2.00 Gy dose range and at the 0.16 Gy/min dose rate (γ -rays contamination did not exceed 8–10%). Experiments in fast neutron RBE determinations were carried out at an exposure of lymphocyte cultures in an air and tissue equivalent phantom.

Lymphocyte cultures were exposed to fast neutrons with 22 MeV average energy in the 0.01–4.00 Gy dose range and at 1.00 Gy/min dose rate (γ -rays contribution did not exceed 5%). Dosimetry of the absorbed dose of fast neutrons was carried out with a Va-R-253 ionisation chamber and a Va-J-18 dosimeter by direct measurements of the charge on the target.

Similar experiments were carried out for ^{60}Co γ -rays for the comparison and as a reference dose to obtain RBE values. An ionisation chamber and a ferrous sulfate dosimeter were used for the dosimetry of the absorbed doses. Lymphocyte cultures were exposed to 0.15–0.30 Gy the dose range of ^{137}Cs γ -rays at the 2.00 Gy/min dose rate and in the 0.3–5.0 Gy dose range of ^{60}Co at the 2.2 Gy/min dose rate.

Lymphocyte cultures were irradiated in G_0 phase of cell cycle [4].

RESULTS

The comparative analysis of chromosome radiosensitivity to ^{60}Co and ^{137}Cs γ -photons demonstrated higher cytogenetic effectiveness of ^{137}Cs and made it possible to determine RBE dose dependence (Figure 1).

The increase in the dose led to the lower RBE values of ^{137}Cs γ -rays which in the range of doses investigated (0.3–5.0 Gy) varied between 3.5 and 1.3, respectively. Thus a difference in RBE values was most accurately determined in the 0.5–2.0 Gy dose range usually used in radiotherapy.

The RBE dose dependence of neutrons (22 MeV) calculated according to the criterion of total chromosome aberrations in lymphocytes is shown in Table 1. In the 0.2–4.0 Gy dose range, RBE decreases from 2.8 to 1.0. The difference in RBE values is most precisely determined in the 0.2–2.0 Gy dose range usually used in radiation therapy.

Figure 2 shows fast neutrons RBE dose dependence. RBE values estimated according to the yields of chromosome aberrations and percent of the cells containing aberrations do not differ but they change for neutrons from 5.2 to 2.6 and 5.7 to 2.1 within the 0.4–2.2 Gy dose range, respectively. Comparative estimation of fast neutron RBE values (Table 1, Figure 2) shows that 6 MeV neutrons give a more effective chromosome aberration yield as compared with 22 MeV neutrons. For example, at 0.5 Gy the RBE value for 22 MeV neutrons is 2.4 and that for 6 MeV neutrons is twice as high. However, at 2.0 Gy the RBE value for 22 MeV energy neutrons is 1.37 and for 6 MeV it is 2.6. This fact confirms the general tendency: the increase in dose and energy leads to a decrease in neutron RBE values. The RBE for

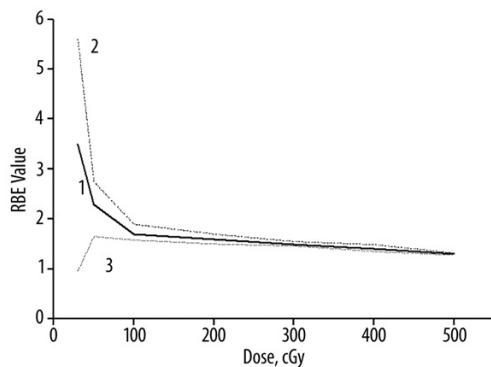


Figure 1. RBE dose dependence of ^{137}Cs γ -rays: 1- RBE average value; 2 and 3 – 95% confidence limits.

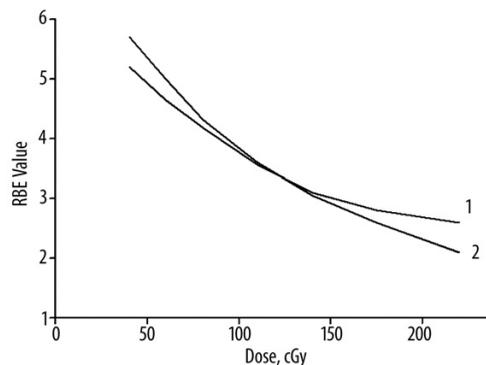


Figure 2. Fast neutron (6 MeV) RBE dependence on dose according to the frequency of chromosome aberrations (1) and damaged cells (2).

Table 1. 22 MeV neutron RBE dose dependence.

Dose, Gy	RBE value
0.2	2.80
0.5	2.44
1.0	1.86
2.0	1.37
3.0	1.13
4.0	1.0

neutrons with different energies takes only an approximate value due to the induction of the “excess” damage when the irradiation dose increases [5].

The application of neutron radiation in radiobiological experiments requires good dosimetry and investigation of neutron beam parameters. Comparative examination of the absorbed dose decrease in air and in a tissue-equivalent material was one of the important tasks of this study. Additionally, during the exposure of large biological objects it is necessary to take into account irregularity of irradiation caused by the attenuation of the primary beam. Given all these facts we studied the dependence of cytogenetic changes caused by 6 MeV energy neutrons in the 0.25–2.20 Gy dose range on the tissue-equivalent phantom depth.

Experimental cytogenetic data indicate the absence of reliable differences in the cytogenetic injury of lymphocytes after neutron irradiation of human lymphocyte culture in phantom (depth of 2.6 and 10.0 cm along the axis of the beam) in comparison with that irradiated in air. It was shown that cytogenetic effectiveness has a tendency to decrease with the increase in the depth of the water phantom. The largest decrease was observed at the depth of up to 6 cm (Figure 3).

DISCUSSION

The following factors specified in the present study indicated higher effectiveness of ^{137}Cs in comparison with ^{60}Co irra-

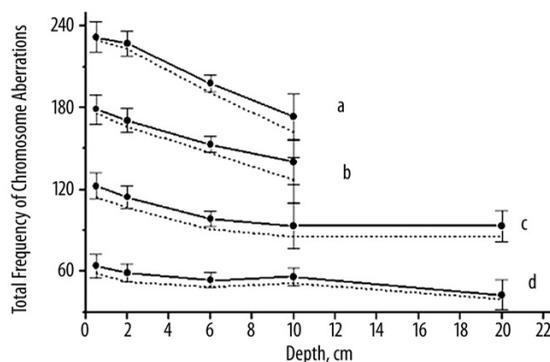


Figure 3. Dependence of neutron effectiveness on the depth of a water phantom according to the total chromosome aberration frequency. Solid line represents neutrons and accompanying γ -rays; dashed line – neutrons only.

diation. First, our radiobiological study was conducted under conditions approximating to the maximum the *in vivo* (clinical) circumstances, i.e. including a steep dose gradient in the irradiated volume. Second, the energy of ^{137}Cs γ -rays is half as low as the energy of ^{60}Co γ -rays. Cytogenetic data on ^{137}Cs RBE values which exceed the value of one in agreement with the investigations made on the basis of human chromosome damage estimations and of the degree of radiation pathomorphosis of the endometrium malignant neoformation [6,7]. Thus made it possible to determine the tendency to lower ^{137}Cs γ -ray effectiveness in comparison with ^{60}Co irradiation.

The interest in the studies of neutron RBE is caused by the need to establish radiation safety norms, to make risk assessment for exposed personnel and to formulate and improve an appropriate protocol for neutron therapy [8]. We have shown that the cytogenetic effectiveness of neutrons has a tendency to decrease with the increase in the phantom depth. This can be due to the filtration of low-energy neutrons at the depth of the irradiated object, which is very important for radiotherapy planning in cases of deeply located tumours. Thus the estimation of neutron effectiveness requires detailed physical and biological dosimetry measurements.

Variogated dependence of chromosome aberration frequencies on the radiation dose and neutron energy that leads to neutron RBE value higher than 1 can be explained by the LET dependence on the energy of irradiation and by the influence of the micro-distribution of absorbed energy in the volume of cell nucleus.

The cytogenetic results obtained showing that the neutron RBE value decreases with the increase in their energy (6, 22 MeV) in the dose range studied are in agreement with the data on RBE estimation of 0.37 and 0.57 MeV neutrons during the exposure of human peripheral blood lymphocyte culture [1]. Similar RBE dependence on neutron energy was shown in the assessment of cell survival and oncogenic transformation of C3H10T1/2 cells exposed to 0.35 MeV and 13.7 MeV neutrons [9]. The data presented in this study were taken into account in neutron therapy planning for deeply located tumours [10, 11] and in intracavitary radiotherapy of patients with malignant endometrium neoplasms [12].

CONCLUSIONS

The results of radiobiological studies based on cytogenetic effects indicate that RBE values of fast neutrons (6 and 22 MeV) depend on their energies and on the depth of the irradiated biological object. The RBE of ^{137}Cs γ -rays estimated according to the frequency of chromosome aberrations in human peripheral blood lymphocytes in a dose range of 0.15–3.00 Gy exceeds 1 and reaches the highest values at lower doses.

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