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ORIGINAL PAPER

Biological efficiency of the Brookhaven Medical Research Reactor mixed neutron beam estimated from gene mutations in *Tradescantia* stamen hair cells assay

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Abstract The relative biological effectiveness (RBE) of low energy neutrons for the induction of various abnormalities in *Tradescantia* stamen hair mutation (Trad-SH) assay was studied using two clones (T-4430 and T-02), heterozygous for flower color. Dose response relationships for gene mutations induced in somatic cells of Trad-SH were investigated after irradiation with a mixed neutron beam of the Brookhaven Medical Research Reactor (BMRR), currently used in a clinical trial of boron neutron capture therapy (BNCT) for glioblastoma. To establish the RBE of the BMRR beam in the induction of various biological end-points in *Tradescantia*, irradiation with various doses of γ-rays was also performed. After irradiation all plants were cultivated several days at Brookhaven National Laboratory (BNL), then transported to Poland for screening the biological end-points. Due to the post-exposure treatment, all plants showed high levels of lethal events and alteration of the cell cycle. Plants of clone 4430 were more reactive to post-treatment conditions, resulting in decreased blooming efficiency that affected the statistics. Slope coefficients estimated from the dose response curves for gene mutation frequencies allowed the evaluation of ranges for the maximal RBE values of the applied beam *vs.* γ-rays as 6.0 and 5.4 for the cells of T-02 and T-4430, respectively. Estimated fraction of doses from neutrons and corresponding biological effects for the clones T-02 and T-4430 allowed to evaluate the RBE values for neutrons part in the beam as 32.3 and 45.4, respectively.

Key words neutrons • relative biological efficiency • *Tradescantia*

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Introduction

Experimental data on the effects of neutron irradiation in the low energy and low doses region are relatively few. Part of the difficulty in the estimate of biological effectiveness lies in the different mechanisms of energy loss for neutrons of various energies. This is not a serious problem for mono-energetic beams of high-energy neutrons, which lose most of the energy through the production of recoil protons, in elastic collisions with hydrogen nuclei. It becomes serious, though, for the broad spectra of fast neutron energies produced by reactors, and it becomes a problem for thermal neutrons, which lose their energy through capture reactions with various elements. The most important of these elements are the isotopes of hydrogen, nitrogen and boron. Each of them forms an unstable isotope which emits a different type of radiation; hydrogen – a γ-ray, a nitrogen isotope emits a proton (plus a β particle later) and boron – the α particle. In addition, the recoil nuclei, such as the lithium nucleus from the $^{10}B(n,\alpha)^{7}Li$ reaction, also deposit some energy in the tissue. This paper presents the results of studies on the relative biological effectiveness of the clinical epithermal neutron beam of the Brookhaven Medical Research Reactor for the induction of various abnormalities in *Tradescantia* stamen hair cells (Trad-SH) using two clones heterozygous for flower color.

Material and methods

Tradescantia plant (*Commelinaceae*) has several unique features that make it useful for radiobiological experiments. Flower cells in clones that are heterozygous for flower color easily mutate from the normal blue color to pink. *Tradescantia* clone 02 is known as the most radiosensitive plant [11]. A relatively short experimental period is required for experiments with this assay. Induced mutations are usually scored during days 11 through 15 after irradiation, because these days usually show the highest frequency for pink mutation. Each flower bears about 300 stamen hairs, and each stamen hair that is the progeny of irradiated cell, consists of about 20 cells.

Its somatic cells have almost the same range of sensitivity as mammalian cells, particularly human lymphocyte [2]. While other eucaryotic systems may also mutate at low doses, *Tradescantia* has a distinct advantage over them, because hundreds of thousands of stamen hairs can be scored easily and quickly. This allows to gather enough data from very low dose irradiation to be statistically significant.

Biological assay

Tradescantia plants were grown in the Institute of Nuclear Physics in Kraków in a glasshouse of the Department of Radiation and Environmental Biology in the conditions previously described [3, 4]. Fresh cuttings of two clones (02 and 4430) of *Tradescantia* plants were transported to BNL where they were grown in aerated water for ten days using an 18 h day light cycle and normal room conditions of humidity. All plants were cultivated for several days after irradiation at the same conditions at Brookhaven National Laboratory (BNL), and then transported to Poland and put into a growth chamber with controlled conditions for light, temperature and humidity. The temperature in the growth chamber ranged between 20.1 and 21.6°C. Nutrient solution (Hogland) was changed about every four days. After transportation, a significant decrease in blooming efficiency was observed that has affected statistics.

Neutron irradiation

Irradiation was done at the Medical Research Reactor at Brookhaven National Laboratory. *Tradescantia* cuttings of two clones T-02 and T-4430 (23 cuttings of each clone per dose point) were placed in a special experimental box (50×30×15 cm) filled with de-ionized water.

Total dose rate estimated at the side position in the box and at a 10 cm distance from the port face was 0.92 cGy/MW×min [7], and it was consisting of:

- 0.05 cGy/MW×min from epithermal neutrons,
- 0.04 cGy/MW×min from fast neutrons and
- 0.83 cGy/MW×min from γ-rays.

Irradiation times varied between 268 and 1606 seconds and calculated dose values for plants situated at the "side" position at a 10 cm distance from the beam window were in the range between 0.02 and 0.12 Gy, respectively.

Gamma irradiation

Cesium-137 was used as the reference source of γ-radiation. Plants inflorescence of both clones: 02 and 4430 (23 cuttings per each dose, the stems shielded with lead) were irradiated at a 80 cm distance from the source for various periods of time. The dose rate of gamma radiation (0.667 MeV energy) at this distance was 0.444 Gy/min. The doses of γ-radiation used for these experiments were 0.3, 0.6 and 0.8 Gy.

Mutation screening

Flowers were harvested at their full blooming, normally early in the morning of each day and stored in a refrigerator until scoring. Stamen hairs were carefully removed from the flowers with forceps and placed on a slide glass in a drop of a mineral oil. Scoring, with the use of a stereo-microscope (×25 magnification), was performed to determine the gene mutation frequency (GF) – characterized by single or numerous adjacent pink cells in the hair. Each pink sector was counted as one mutational event [3]. Because of the low number of flowers blooming in the standard scoring period, to improve the statistics the screening period was extended from day 9 to day 20. The mean values of mutation frequency calculated for the scoring period and expressed as the number of mutations in 100 cells, were used as a measure of the mutation effect caused by the exposure.

Time of irradiation [s]	Dose [Gy]	NOF (days 11–15)	NOF (days 9–20)	NOH (days 9–20)	GF × 10^2 ± SE
0	0	14	28	4608	0.04 ± 0.03
268	0.02	12	27	5545	0.23 ± 0.06
535	0.04	8	13	2565	0.6 ± 0.2
803	0.06	11	21	3150	0.7 ± 0.2
1070	0.08	12	25	4435	0.8 ± 0.1
1338	0.10	5	15	3666	1.1 ± 0.2
1606	0.12	9	20	4608	1.3 ± 0.3

Table 1. Results of gene mutation frequencies (GF) measured in T-02 cuttings irradiated with a mixed beam from the BMRR.

NOF – number of flowers, NOH – number of hairs, GF – number of gene mutations per cell

Time of irradiation [s]	Dose [Gy]	NOF (days 11–15)	NOF (days 9–20)	NOH (days 9–20)	GF × 10^2 ± SE
0	0	18*	-	4284	0.3 ± 0.2
268	0.02	3	7	1698	0.6 ± 0.2
535	0.04	1	4	426	0.7 ± 0.2
1070	0.08	1	2	3150	0.7 ± 0.1
1338	0.10	2	6	528	1.7 ± 0.7

Table 2. Results of gene mutation frequencies (GF) mutation frequencies in 100 cells measured in T-4430 cuttings irradiated with a mixed beam from the BMRR.

NOF – number of flowers, NOH – number of hairs, * – number of flowers screen from the plantation before irradiation, GF – number of gene mutations per cell

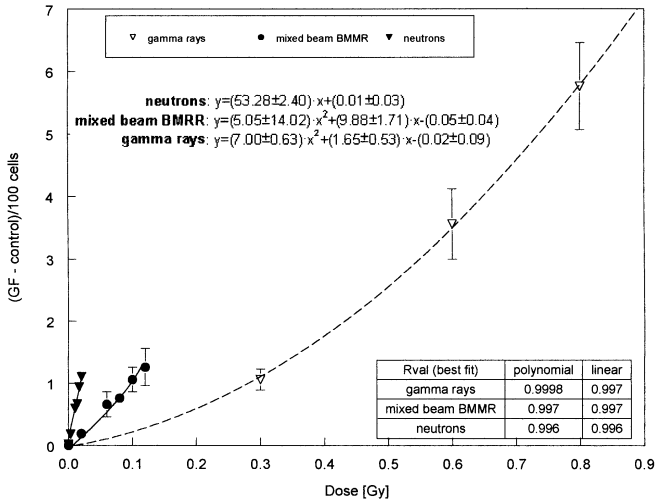

Fig. 1. Gene mutation frequencies (±SE) for T-02 irradiated with a mixed beam BMRR and gamma rays.

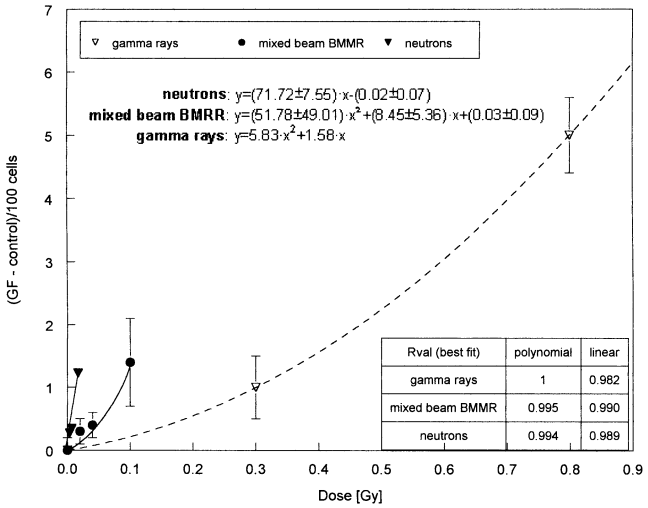

Fig. 2. Gene mutation frequencies (±SE) for T-4430 irradiated with a mixed beam BMRR and gamma rays.

Results

Table 1 shows length of irradiation and consequent total doses applied to irradiation of T-02 cuttings with a therapeutic beam from BMRR. In Table 1 is also shown the number of stamen hairs (NOH) that represents the number of the analyzed, for the presence of mutational events, progenies of irradiated cell and gene mutation frequencies (GF) estimated from the whole screening period. Similarly, Table 2 shows results obtained from T-4430 cuttings after irradiation with the same beam. Tables 3 and 4 show values of the total number of stamen hairs analyzed and gene mutation frequencies induced in the stamen hair cells of clones T-02 and T-4430, respectively, by irradiation with various doses of Cs-137 gamma rays. Figs. 1 and 2 present dose-response relationships for gene mutation frequencies induced by irradiation with the mixed BMRR beam and by Cs-137 γ-radiation in clones T-02 and T-4430, respectively. There are also shown parameters describing goodness of the best fit. Dose response curves obtained after both types of irradiation (BMRR therapeutic beam and gamma radiation) are better described by linear quadratic formulas. Respective equations are also presented in Figures. Different efficiencies of both types of irradiation are expressed by relative biological effectiveness (RBE). According to the molecular theory of radiation biology [8] a maximum value of the experimentally determined RBE is the value that operates at low doses and is defined as:

$$RBE_0 = \alpha_n/\alpha_\gamma,$$

where α are the slope coefficients from the dose response curves for tested and standard radiation (gamma). The maximal value (RBE_0) is therefore the parameter which is of direct relevance to the Quality Factor (Q). Table 5 contains slope coefficients estimated from dose response curves estimated for both clones and the maximal RBE values of the BMRR beam under the study *vs.* γ-rays. The extension of scoring period might affect the RBE estimate. Fig. 3 presents a comparison of dose response relationships estimated from the means of 3 days of maximal values obtained for the gene mutations induced by epithermal neutrons or γ irradiation groups for clone T-02 and for the whole 12-day scoring period. There is no statistically significant difference in the ratio of slope coefficients between linear fits for 3 days *vs.* the 12-day scoring periods.

Discussion

Relative biological effectiveness of the investigated beam (RBE values of 5.4 for T-4430 and 6.0 for T-02), calculated from

Dose [Gy]	NOF (days 11–15)	NOF (days 9–20)	NOH (days 9–20)	GF × 10^2 ± SE
0	14	28	4608	0.04 ± 0.03
0.3	7	28	7264	1.04 ± 0.17
0.6	1	13	2851	3.54 ± 0.56
0.8	5	16	3381	5.76 ± 0.70

Table 3. Results of gene mutation frequencies (GF); mutation frequencies in 100 cells measured in T-02 cuttings irradiated with γ-rays.

NOF – number of flowers, NOH – number of hairs, GF – number of gene mutations per cell

Dose [Gy]	NOF (days 11–15)	NOF (days 9–20)	NOH (days 9–20)	GF × 10^2 ± SE
0	18*	-	4284	0.3 ± 0.1
0.3	1	6	1254	1.3 ± 0.5
0.6	4	9	1968	5.0 ± 0.4
0.8	1	11	2574	5.3 ± 0.6

Table 4. Results of gene mutation frequencies (GF); mutation frequencies in 100 cells measured in T-4430 cuttings irradiated with γ-rays.

NOF – number of flowers, NOH – number of hairs, * – number of flowers screen from the plantation before irradiation, GF – number of gene mutations per cell

Table 5. Estimated fraction of doses from neutrons and corresponding biological effects (GF × 10^2) for the clones T-02 and T-4430.

Total dose [Gy]	Neutron dose [Gy]	Biological effects					
		T-02			T-4430		
		Mixed beam BMRR	Gamma*	Neutrons	Mixed beam BMRR	Gamma*	Neutrons
0	0	0	0	0	0	0	0
0.02	0.003	0.19	0.01	0.18	0.30	0.03	0.27
0.04	0.007	-	-	-	0.40	0.06	0.34
0.06	0.010	0.66	0.06	0.60	-	-	-
0.08	0.014	0.76	0.09	0.67	-	-	-
0.10	0.017	1.06	0.12	0.94	1.40	0.17	1.23
0.12	0.020	1.26	0.15	1.11	-	-	-

* – gamma effect is estimated from dose-response curve for γ-rays presented in Figs. 1 and 2.

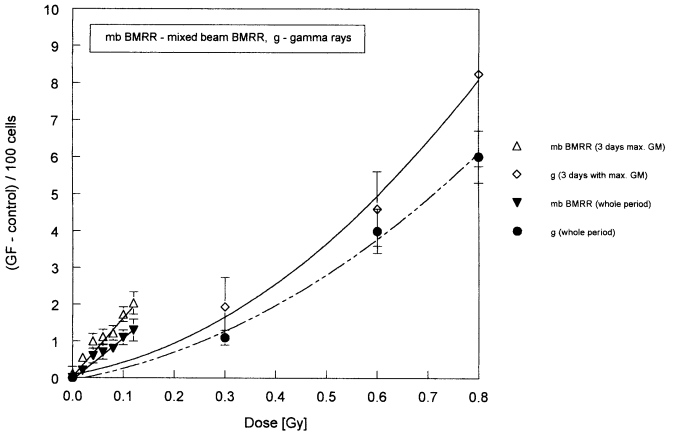

Fig. 3. Gene mutation frequencies (±SE) for T-02 irradiated with a mixed beam BMRR and gamma rays estimated for 3 days with maximal GM values in comparison with the whole scoring period.

presented results does not seem to be low, particularly, that it has to consider the low per cent of fast and epithermal neutrons in the mixed beam (~10% – see Material and Methods). From the results presented in this paper, assuming the composition of the investigated beam and knowing the efficiency of gamma rays in the induction of gene mutations in Trad-SH cells under the same experimental conditions (Tables 3 and 4), we can evaluate biological impact from neutrons. Table 5 shows fractions (of the total doses) delivered to biological samples (both clones respectively) by neutrons, and the biological effects corresponding to them, estimated after subtraction from the total effects detected, those due to the gamma component. In Figs. 1 and 2 are shown calculated effects for both clones respectively, corresponding to the neutrons doses. Table 6 presents α_{PF} coefficients, and maximal RBE values (45.4 for clone T-4430, and 32.3 for clone T-02) estimated for the fraction of neutrons from the investigated beam.

The obtained values of RBE_{max} seem to be in good agreement with the expectation based on the biological efficiency dependence on the neutron energy. For various types of mutations in *Tradescantia* stamen hairs a linear relationship was shown at low doses of various energy neutrons, and variation in RBE values showing that the RBE depends not only on the dose but also on the energy of neutrons and biological end-point chosen. Obtained by us here RBE values are higher than those measured previously for fast neutrons from U-120 and from ^{252}Cf [3–6]. The evaluated RBE value is also higher than the RBE that had been reported for more energetic neutrons; 4 and 9 for 5.6 MeV and fission neutrons, respectively (Cebulska-Wasilewska et al. [5, 6]), or 12 for 32.5 MeV neutrons (Pihet et al. [10]). The neutron energy distribution in the BMRR neutron beam was estimated as follows: about 50% of the neutron dose were from epithermal neutrons (0.24 – 10 keV), and the rest was coming from fast neutrons with energy E_n>10 keV [7]. According to Kappas et al. [9] maximal RBE values for gene mutations in *Tradescantia* (clone 4405) was 93 in case of the monoenergetic neutrons (0.4 MeV) and 250-kVp X-rays. From the microdosimetric characteristics of monoenergetic neutron beams the highest maximum of RBE should also be expected at the energy close to 0.3 MeV [2]. The RBE value presented in this paper is lower than values reported and expected for more effective energies. Using somatic mutation in the stamen hair of *Tradescantia* flowers Davies and Bateman (quoted after Bender [1]) determined the relative effectiveness of 0.65 MeV fast neutrons from the ^{3}H (p,n) ^{3}He reaction as compared with X-rays. At the arbitrary level of 15 mutations per flower, the RBE was about 17.5. Davies and Bateman calculated the maximum RBE to be about 40 (for chronic exposures), where the low LET dose-square component would be minimized. RBE values for pink mutation frequency for low energy neutrons were also reported by Underbrink et al. [12] and by Kappas et al. [9]. The highest values for the RBE of neutrons (60–140), for various biological end-points in *Tradescantia* were estimated by them [5]. However, for pink events frequency in T-02 induced by 0.43 MeV neutrons and 250 kV X-rays they evaluated RBE = 31.3 at the very low dose (0.4 cGy). A number of radiobiological experiments presented by Bewley [2] confirm that one of the highest RBE values is observed for gene mutations in Trad-SH

Table 6. Coefficients α and values of the RBE, estimated from dose response curves (Figs. 1 and 2) for gene mutations frequency experimental and reevaluated for neutrons in the beam.

	α coefficients (±SE)			RBE (±SE)	
	Mixed beam BMRR	Neutrons	Gamma	Mixed beam BMRR	Neutrons
T-4430	8.5 ± 5.4	71.7 ± 7.8	1.6 ± 0.0	5.4 ± 3.40	45.4 ± 4.9
T-02	9.9 ± 1.7	53.3 ± 2.4	1.7 ± 0.5	6.0 ± 1.92	32.3 ± 11.8

cells (~40) should be close to 0.3 MeV, although the calculation are made considering a monoenergetic beam of neutrons. According to this and to the results of Underbrink [12], our evaluation, although in case of *Tradescantia* 4430 gives results slightly overestimated, in general, it confirms the RBE neutron energy dependence.

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