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Dose Estimations of Fast Neutrons from a Nuclear Reactor by Micronuclear Yields in Onion Seedlings

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Irradiations of onion seedlings with fission neutrons from bare, Pb-moderated, and Fe-moderated ^{252}Cf sources induced micronuclei in the root-tip cells at similar rates. The rate per cGy averaged for the three sources, $<b_n>$, was 19 times higher than rate induced by ^{60}Co γ-rays. When neutron doses, D_n, were estimated from frequencies of micronuclei induced in onion seedlings after exposure to neutron-γ mixed radiation from a 1 W nuclear reactor, using the reciprocal of $<b_n>$ as conversion factor, resulting D_n values agreed within 10% with doses measured with paired ionizing chambers. This excellent agreement was achieved by the high sensitivity of the onion system to fast neutrons relative to γ-rays and the high contribution of fast neutrons to the total dose of mixed radiation in the reactor's field.

INTRODUCTION

For studying the unique biological effectiveness of fission neutrons from nuclear reactors, separate dosimetry of neutrons and γ -rays in mixed radiation from reactors is essential[1]. Dosimetric studies of mixed radiation from the Kinki University nuclear reactor have shown that plastic nuclear track detectors, TS16N, and paired Fricke solutions made of light and heavy water, as well as paired ionizing chambers[1], can be used to determine tissue absorbed dose of fast fission neutrons separately from γ-ray dose[2–4]. Nevertheless, we sought a novel method for neutron dosimetry in the reactor's field, because the track detectors and the paired chambers are not easy to use properly for biologists, and the Fricke dosimeter requires several tens of hours for irradiation in the mixed field to obtain reliable measurements due to low dose rate of fast

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neutrons in the field[4].

Evans and coworkers[5] reported that irradiation of *Vicia faba* seedlings with 3 MeV neutrons produced micronuclei, namely, small sized, excess nuclei resulting from chromosome damage, in the root-tip cells with a 10.5-fold higher efficiency than γ-rays. If relative biological effectiveness (RBE) of fast neutrons to γ-rays is actually so large, we can measure neutron doses in mixed radiation fields, with reasonable accuracy, by measurements of micronuclear yields. This idea was tested in the present study using onion seedlings. The choice of onion seedlings was suggested by the relatively simple methodology of micronucleus assay developed by Hanmoto[6,7]. Here we report that, for producing micronuclei in the root-tip cells, onion seedlings were about 20 times more sensitive to ^{252}Cf neutrons having mean energies of 1.0 to 2.1 MeV than to ^{60}Co γ-rays, and that the calibrated onion system could be used to estimate accurately doses of fast neutrons in the mixed radiation field of the Kinki University reactor.

MATERIALS AND METHODS

Onion seedlings

Onion (*Allium cepa* L.) seeds of variety OK Yellow (Takii Seed Co., Kyoto, Japan) were seeded on paper beds soaked with distilled water (DW) and allowed to germinate at 25°C on the beds. Resulting seedlings with roots of about 5 mm were sampled 48 h later and transferred onto freshly prepared DW-soaked paper beds in 60 × 15 mm or 30 × 13 mm polystyrene dishes. Seedlings in the covered dishes were irradiated with neutron-γ mixed radiation or γ-rays.

Irradiation

The neutron source used for the calibration of onion seedlings is a ^{252}Cf source at Hiroshima University[8]. To obtain three different energy spectra of fission neutrons, this source was used firstly without moderation and then with 5 cm Pb or 10 cm Fe moderator. For each irradiation, dosimetry of the mixed field of neutrons and γ-rays was done using paired ionizing chambers[8]. One of the chambers was made of tissue equivalent plastic and the other was of graphite. The former was used with tissue-equivalent gas and the latter with CO_2, both with a flow rate of 12 cc/min. Dose rates used for neutron-γ mixed radiation from the bare source, the Pb-moderated source, and the Fe-moderated source were 1.5, 0.14, and 0.12 cGy/min, respectively; fractions of neutron doses in the total doses were 66%, 93%, and 91%, respectively.

For γ irradiation, we used a standard ^{60}Co source at Hiroshima University. The dose rate used was 43 cGy/min as measured by the tertiary standard Japanese Radiological Physicists (JARP) chamber[8].

The Kinki University nuclear reactor used for biodosimetry of fast neutrons is called the University Teaching and Research Reactor, type B. The nominal output power is 1 watt. It has two cores separated by 46 cm internal graphite, each of which contains ^{235}U-enriched uranium fuels immersed in a small quantity of light water. At the center of the internal graphite, a graphite stringer of 9.6 × 9.6 cm square and 122 cm long can be withdrawn to provide a cavity for sample irradiation. For biodosimetry, the 60 × 15 mm dishes containing onion seedlings were placed at the center of the core height in the cavity, and the reactor was operated at 1 watt. Graded doses of neutron-γ mixed radiation were given to the samples by changing the irradiation period.

Micronucleus assay

Irradiated seedlings were cultured at 25°C for 24 h, approximate time for one mitotic cycle of onion meristematic cells[9,10]; the roots were fixed, stained and macerated in a 7:3 mixture of acetic dahlia and 1 N HCl for 15 min[6,7]. The dye solution was prepared by dissolving a 0. 5 g sample of dahlia violet (Wako Pure Chemical, Osaka, Japan) into 100 ml of 30% acetate. The treated roots were briefly washed by DW; terminal 1–2 mm of the roots were mounted using 50% glycerin as medium and squashed on slides, one root each. Semi-permanent preparations made in this way were microscopically inspected at a 400× magnification for the presence of more than one nucleus in the meristematic cells in interphase. The additional nuclei, which were smaller than the normal, were scored as micronuclei.

Frequency of micronuclei was calculated as the number of micronuclei detected per 100 interphase cells observed. The frequency was determined for each irradiation as the weighted mean for 5–10 slides, on each of which 500 or more interphase cells were observed.

RESULTS

Induced rate of micronuclei by ^{252}Cf neutrons and RBE to γ -rays

As seen in Fig. 1, frequency of micronuclei, F(%), increased linearly with dose, D (cGy), of neutron-γ mixed radiation from ^{252}Cf source and γ-rays from ^{60}Co source as follows:

$$F = a+bD = (0.10 \pm 0.03)+bD \quad (1)$$

where a is spontaneous frequency and b induced rate of micronuclei (/100 cells/cGy). The a value (±1SD) in the second form of Eq. (1) was obtained as average of concurrent controls for 13 experimental runs in the present study. Experimentally determined b values for neutron-γ mixed radiation, b_{ng}, and γ-rays, b_g, are 0.78 ± 0.18 and 0.063 ± 0.011, respectively.

If fraction p of a total dose of neutron-γ mixed radiation is contributed by fast neutrons, the b_{ng} can be approximated by

$$b_{ng} = pb_n + (1\text{-}p)\, b_g \quad (2)$$

Assuming that b_g for ^{252}Cf is equal to b_g for ^{60}Co, from Eq.(2), we obtained b_n value of 1.15 ± 0.27 for experimental p value of 0.66 (Table 1).

In Fig. 2, frequencies of micronuclei observed after irradiation with ^{252}Cf radiation through 5 cm Pb and 10 cm Fe moderators are plotted against neutron doses; the fraction of total dose contributed by contaminating γ-rays was negligibly low for either irradiation. The two dose

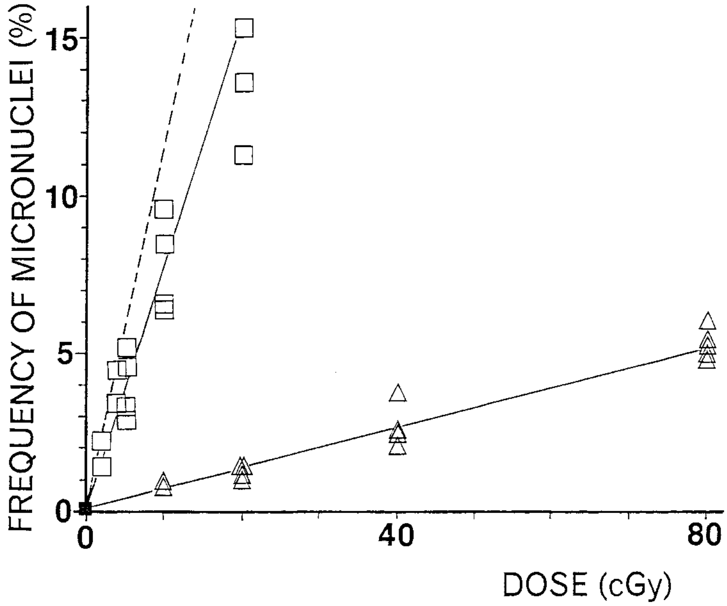

Fig. 1. Frequencies of micronuclei induced in onion seedlings plotted against doses of neutron-γ mixed radiation from bare ^{252}Cf source (□) and γ -rays from ^{60}Co source (△). Solid square represents spontaneous frequency. Dotted line is theoretical dose response to fast neutrons constructed using induced rate of micronuclei by neutron-γ mixed radiation after correction for contribution by contaminated γ -rays.

Table 1. Induced rates of micronuclei in onion seedlings by γ -rays from ^{60}Co source and fast neutrons from bare and moderated ^{252}Cf sources

Source	Mean energy[a] (MeV)	Induced rate[b], b_g, b_n (/100 cells/cGy)	RBE[b] (b_n/b_g)
^{60}Co		0.063 ± 0.011	1
^{252}Cf	2.1	1.15 ± 0.27	18 ± 5
^{252}Cf (5 cm Pb)[c]	1.7	1.20 ± 0.23	19 ± 4
^{252}Cf (10 cm Fe)	1.0	1.24 ± 0.08	20 ± 2
^{252}Cf, averaged		1.20 ± 0.05	19 ± 1

[a] Calculated with MCNP-4A code[11)]
[b] Error is 1SD.
[c] Moderator used

response relations followed Eq.(1), where b_n values of 1.20 ± 0.23 and 1.24 ± 0.08 were obtained for the Pb-moderated and the Fe-moderated sources, respectively (Table 1). Within the limits of experimental error, these b_n values are in good agreement with that obtained for neutrons from the bare ^{252}Cf source.

RBE values obtained as the ratios of b_n to b_g for the bare, the Pb-moderated and the

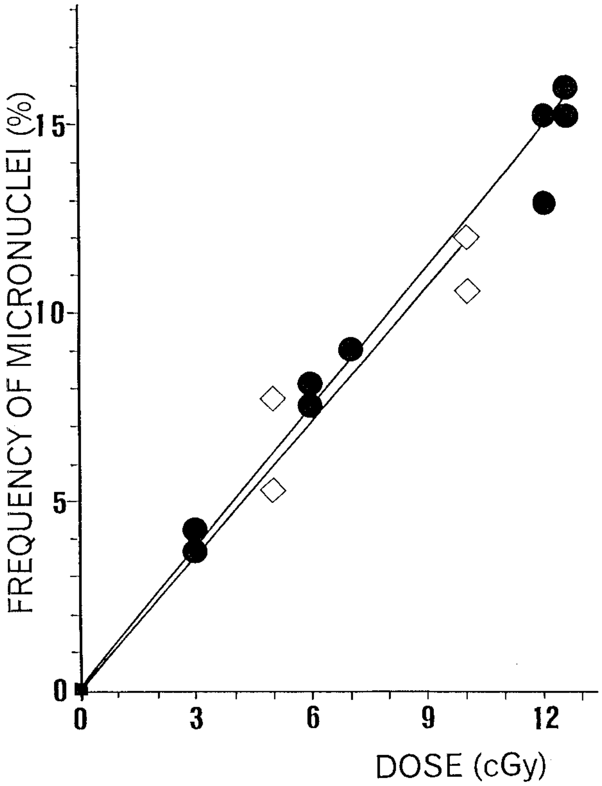

Fig. 2. Frequencies of micronuclei induced in onion seedlings plotted against doses of fast neutrons from 5 cm Pb-moderated ^{252}Cf source (◇) and 10 cm Fe-moderated ^{252}Cf source (●). Solid square represents spontaneous frequency.

Fe-moderated sources are 18 ± 5, 19 ± 4 and 20 ± 2, respectively (Table 1).

Biodosimetry of fast neutrons in a neutron-gamma mixed field

Using the average of the three b_n values (Table 1), $<b_n>$, and the spontaneous frequency of 0.10%, we formulated the following equation for biological measurements of neutron dose, D_n, in neutron-γ mixed radiation:

$$D_n = (F\text{-}a)/<b_n> = (F\text{-}0.10)/1.20 \tag{3}$$

As seen in Table 2, F value recorded after irradiation of onion seedlings in the neutron-γ mixed field of the Kinki University reactor varied depending on the exposure time. Using Eq.(3), we estimated the neutron dose D_n for each of the F values (Table 2). For comparison, we measured dose rates of fast neutrons and γ-rays in the same field with a polyethylene-walled, ethylene filled ionizing chamber and a graphite-walled, CO_2 filled chamber, IC-17P and IC-17G (Far

Table 2. Dosimetry of fast neutrons at the center of the Kinki University nuclear reactor operated at 1 watt

Exposure time (min)	Onion dosimeter F(%)[a]	Onion dosimeter D_n(cGy)[b]	Ion chambers D_n(cGy)	Relative diff., R(%)[c]
10	4.46 ± 0.33	3.6	3.4	+6
20	8.09 ± 0.47	6.7	6.9	–3
30	12.18 ± 0.41	10.1	10.3	–2
60	23.70 ± 1.57	19.7	20.6	–4

[a] Frequency ± 1SD of micronuclei averaged for 2 or 3 independent irradiations.
[b] Dose value obtained by substituting F value in Eq.(3) in text.
[c] $R = \{(D_n$ by onion dosimeter $-D_n$ by ion chambers$)/D_n$ by ion chambers$\} \times 100$.

West Tech. Inc., California); the rates were 20.6 and 19.6 cGy/h, respectively. D_n derived from the measured dose rate of fast neutrons for each exposure time is shown in the fourth column of Table 2. As seen in Table 2, D_n values estimated by the onion dosimeter are in good agreement with those by the chambers.

DISCUSSION

As seen in Table 1, irradiations of onion seedlings with ^{252}Cf fission neutrons having mean energies of 1.0, 1.7, and 2.1 MeV induced micronuclei at similar rates; the rate averaged for the three different spectra, $\langle b_n \rangle$, is 19 times higher than b_g for ^{60}Co. These results support the use of excess frequencies of micronuclei, F-a, by neutron-γ mixed radiation as readings of neutron doses, D_n, and the reciprocal of $\langle b_n \rangle$ as conversion factor for F-a, as shown by Eq.(3).

When neutron doses were estimated using Eq.(3) after irradiations of onion roots for 10, 20, 30, and 60 min in the neutron-γ mixed field of the Kinki University reactor, resultant D_n values in cGy unit for these exposure periods were 3.6, 6.7, 10.1, and 19.7, respectively. As shown by R values in the last column of Table 2, errors in these estimations to doses measured with paired ionizing chambers are as small as + 6, –3, –2, and –4%, respectively. We thus could accurately determine neutron doses in the reactor's mixed field at the order of 10 cGy by measurements of micronuclear yields in onion seedlings.

The good performance of the onion dosimeter was achieved not only by its high sensitivity to fast neutrons relative to γ-rays, but also by the high contribution of fast neutrons to total dose of mixed radiation in the reactor's field, i. e., $p = 0.51$. The reasoning follows: If we assume no experimental error, the relative difference, R, between D_n by the onion system and D_n by paired chambers is theoretically equivalent to the ratio of frequency of micronuclei by γ-rays to that by fast neutrons at a total dose, D, of mixed radiation that is given by

$$R = b_g\,(1\text{-}p)D/b_n pD = (1\text{-}p)/rp, \qquad (4)$$

where r (RBE value) can be substituted by the relative neutron sensitivity of onion dosimeter

(i. e., 19 as the ratio of <b_n> to b_g). Thus, as the error intrinsic to the dose estimation by Eq.(3), Eq.(4) gives R value of +5% for the p value of 0.51. It is thought that the intrinsic error plus experimental error resulted in the empirical R values of −4 to +6%. If p were as low as 0.1, empirical R would have certainly fluctuated around an intrinsic error value of 50%.

In a mixed field with such a low p value, a filter made of γ -ray shielding material may be used to ensure reliable measurements of neutron doses by the onion dosimeter. Otherwise, the dosimeter would provide doses of mixed radiation in Sv unit if we use the reciprocal of b_g, instead of <b_n> , as conversion factor for the readings. The ratio of <b_n> to b_g is close to ICRP's *radiation weighing factor* of 20 for 0.1 to 2 MeV neutrons[12].

In summary, the present study has confirmed the large RBE of fast neutrons to γ -rays for producing micronuclei in plant somatic cells[5] and demonstrated the usefulness of micronuclear yields in onion seedlings as readings of neutron doses in neutron-γ mixed radiation from the Kinki University reactor. In order to evaluate the onion system as a sensitive and reliable dosimeter of fast neutrons in nuclear reactors, however, further studies are needed on the sensitivity to neutrons having mean energies other than those used in the present study and also on the performance in other mixed-field situations. Deployment of the onion system as an economic adjunctive to expensive instruments for radioprotection practices may also merit serious consideration in further studies.

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