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Low-dose neutron dose response of zebrafish embryos obtained from the Neutron exposure Accelerator System for Biological Effect Experiments (NASBEE) facility

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HIGHLIGHTS

- Neutron dose response was determined for embryos of the zebrafish, *Danio rerio*.
- Neutron doses of 0.6, 1 and 2.5 mGy led to neutron hormetic effects.
- Neutron doses of 70 and 100 mGy accompanied by gamma rays led to gamma-ray hormesis.

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ABSTRACT

The dose response of embryos of the zebrafish, *Danio rerio*, irradiated at 5 h post fertilization (hpf) by 2-MeV neutrons with $\leq$ 100 mGy was determined. The neutron irradiations were made at the Neutron exposure Accelerator System for Biological Effect Experiments (NASBEE) facility in the National Institute of Radiological Sciences (NIRS), Chiba, Japan. A total of 10 neutron doses ranging from 0.6 to 100 mGy were employed (with a gamma-ray contribution of 14% to the total dose), and the biological effects were studied through quantification of apoptosis at 25 hpf. The responses for neutron doses of 10, 20, 25, and 50 mGy approximately fitted on a straight line, while those for neutron doses of 0.6, 1 and 2.5 mGy exhibited neutron hormetic effects. As such, hormetic responses were generically developed by different kinds of ionizing radiations with different linear energy transfer (LET) values. The responses for neutron doses of 70 and 100 mGy were significantly below the lower 95% confidence band of the best-fit line, which strongly suggested the presence of gamma-ray hormesis.

1. Introduction

For the general public, the most significant exposure to neutrons derives from the cosmic radiation. People at higher risks of neutron exposures include nuclear reactor workers, well loggers, airline crew members, medical doctors and patients involved in clinical radiotherapy, and astronauts. At high altitudes, neutron exposures become more significant, and the radiation risks associated with high-altitude flights have been studied by National Council on Radiation Protection and Measurements (NCRP) (NCRP, 1995). Medical doctors and patients can be exposed to neutrons during neutron capture therapy or megavoltage X-ray radiotherapy. For astronauts, a major radiation hazard comes from secondary neutrons which contribute to ~10 to 30% of their received radiation inside the spacecraft. As such, it is pertinent to have a good understanding on the radiobiological effects of neutrons. Such knowledge will also be pivotal to the success and effectiveness of neutron therapy.

It has now been well established that neutrons are more effective than photons in causing various biological effects (Hall et al., 1975; Spotheim-Maurizot et al., 1990; Komatsu et al., 1993;

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Vral et al., 1994; Hill et al., 1988; Wolf et al., 2000). A peculiar feature of neutron irradiation is that it is commonly contaminated by a contribution from gamma radiation. In order to focus on the effects of neutrons, researchers have designed neutron irradiation sources in such a way that the majority of the associated gamma radiation is attenuated. For example, in their studies on the radiation induced bystander effect (RIBE) induced by neutrons in human skin keratinocytes, Liu et al., (2006) employed neutrons produced through the ^{7}Li(p,n)^{7}Be reaction using 2.30 MeV protons, and placed a shielding material between the lithium target and the irradiation site to reduce the gamma rays produced through the competing ^{7}Li(p,p′) reaction. Furthermore, the authors purposely employed irradiation conditions to make sure that the associated gamma radiation remained below the threshold dose between 2 and 3 mGy which were already known to be able to induce bystander effects.

However, there was a complication that the low-dose gamma rays could lead to gamma radiation hormesis (or photon radiation hormesis). Hormetic responses are characterized by biphasic dose-response relationships showing a low-dose stimulation and a high-dose inhibition (Calabrese and Baldwin, 2002; Calabrese and Linda, 2003; Calabrese, 2008). Hormetic zones and toxic zones are defined as the zones in which the responses are below the background values for zero doses (i.e., hormesis) and above the background values, respectively. Specifically, photon radiation hormesis refers to the phenomenon where the biological effect of an ionizing radiation (other than photons) is suppressed by a simultaneous small photon radiation exposure (Rithidech and Scott, 2008). For example, it was proposed that a small gamma-ray dose could suppress the lung cancers induced by alpha-particle irradiation (e.g., Refs. Scott, 2008; Scott et al., 2008). In particular, gamma-ray doses from 1 to 2 mGy seemed to have suppressed lung-cancer induction in Wistar rats after they inhaled ^{239}Pu (an alpha-particle emitter) in an insoluble dioxide form, with doses from alpha particles up to ~600 mGy (Scott, 2008; Scott et al., 2008). The proposed mechanisms underlying the gamma-ray hormesis included high-fidelity DNA repair and removal of aberrant cells through apoptosis (Scott and Di Palma, 2006; Bauer, 2007; Portess et al., 2007).

Rithidech and Scott (2008) were the first to demonstrate the contribution of gamma-ray hormesis to low-dose neutron irradiations in terms of reduced frequency of micronucleated cells among irradiated human lymphocytes. The authors used data from human lymphocytes irradiated with mono-energetic neutron sources with five different energies, namely, 0.22, 0.44, 1.5, 5.9 and 13.7 MeV. The associated gamma-ray doses for these sources were estimated to be about 1%, 1%, 2%, 6%, and 6%, respectively, of the neutron doses. For each neutron energy, three different total absorbed doses were employed, namely, 10, 50 and 100 mGy. Rithidech and Scott (2008) used the protection factor (*PROFAC* (Scott and Di Palma, 2006)) to quantify the suppression of neutron-induced biological effects by gamma-ray hormesis, where *PROFAC* = [1 – (observed micronucleated cells)/(expected micronucleated cells)], and the numbers of micronucleated cells were employed as a measure of chromosome damages in the cells. Rithidech and Scott (2008) suggested that addition of a gamma-ray dose of 0.1–0.5 mGy to a total radiation dose of 10 mGy gave *PROFAC* values of 0.52–0.74, and thereby confirming the presence of gamma-ray hormesis.

The discovery of gamma-ray hormesis by Rithidech and Scott (2008) has far-reaching implications on the risk assessment for low-dose neutron exposures, and as such further studies are warranted. For simplification, we only focused on neutrons with a single energy and examined the features of gamma-ray hormesis from the low-dose neutron dose response relationship (i.e., effect vs neutron dose). We used the 2-MeV neutrons delivered by the Neutron exposure Accelerator System for Biological Effect Experiments (NASBEE) facility in the National Institute of Radiological Sciences (NIRS), Chiba, Japan to provide the neutron irradiations (Suda et al., 2009).

We examined the biological effect of different neutron doses on zebrafish (*Danio rerio*) embryos through quantification of apoptosis on the entire embryo. Embryos of the zebrafish were employed as the *in vivo* vertebrate model in the present study. The zebrafish and the embryos have been widely used in studies on the biological effects of ionizing radiation (e.g., refs. Bladen et al., 2005; Geiger et al., 2006; Daroczi et al., 2006; Mothersill et al., 2007; Yum et al., 2007; Choi and Yu, 2015; Choi et al., 2010a, 2010b, 2012a', 2013a', 2013b; Kong et al., 2014) due to its rapid development, fecundity and the fact that zebrafish and human genomes share considerable homology, including conservation of most DNA repair-related genes (Barbazuk et al., 2000).

2. Materials and methods

2.1. Neutron irradiation facility

The NASBEE facility at NIRS was employed to provide the neutron exposure in the present project. NASBEE is a Tandetron™ accelerator (High Voltage Engineering Europa (HVEE) equipped with a multi-cusp ion source (Suda et al., 2009). Neutron beams were produced by bombarding 4-MeV deuterons onto the Be target. A shutter was placed at the beam port to attenuate the gamma rays to keep the gamma-ray contamination in the neutron beam as low as 14% (Suda et al., 2009). Neutrons with an average energy of 2 MeV and a dose rate 220 mGy/h were employed.

2.2. Experimental animals

Adult zebrafish were kindly provided as gifts from RIKEN Brain Science Institute, JAPAN (courtesy Prof. Hitoshi Okamoto). Adult zebrafish of both genders were mixed and kept in a glass tank with 45 L water and kept in an indoor environment with an ambient temperature of 28 °C. The fish were maintained under a 14/10 h light-dark cycle to ensure a good production of embryos. The fish were fed four times a day with commercial tropical fish food (TetraMin, Melle, Germany) or brine shrimp (Brine Shrimp Direct, Ogden, Utah, USA). Spawning of embryos was induced at the beginning of the 14 h light period. The embryos were collected within 15–30 min after the 14-h photoperiod began. This was to ensure the synchronization of the developmental stages of embryos. The collected embryos were then kept in clean water and transferred to an incubator (PIC-100, As ONE Corporation, Osaka, Japan) of 28 °C, which was the most suitable temperature for healthy development, until 4 hour post fertilization (hpf). At 4 hpf, healthy developing zebrafish embryos were selected under a stereomicroscope (Nikon, Chiyoda-ku, Tokyo, Japan) and transferred into a new Petri dish with a layer of agarose (Invitrogen, Life Technologies Corporation, Carlsbad, CA, USA) gel at the bottom (1 mm thick) and 2.5 ml E3 medium (5 mM NaCl, 0.17 mM KCl, 0.33 mM $CaCl_2$, 0.33 mM $MgSO_4$, 0.1% methylene blue), for dechorionation.

2.3. Experimental setup

A total of 10 neutron doses ranging from 0.6 to 100 mGy (namely, 0.6, 1, 2.5, 5, 10, 20, 25, 50, 70 and 100 mGy) were employed in the present work to study the biological effects of neutrons on zebrafish embryos. One of the main objectives was to identify, if any, the gamma-ray hormesis from the dose-response relationship. For each neutron dose, at least two independent

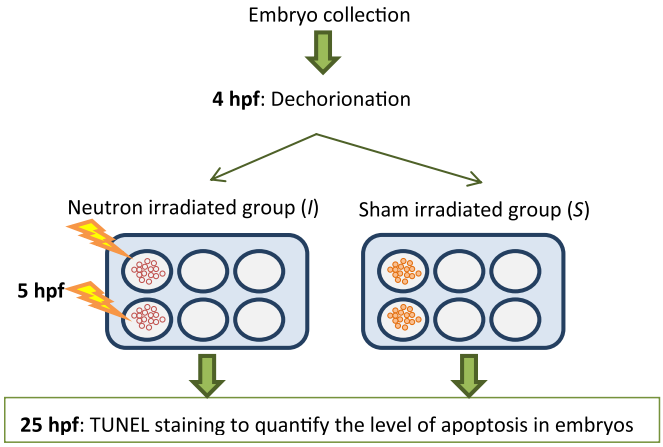

Fig. 1. Schematic diagram showing the procedures for studying the neutron dose response on zebrafish embryos.

experiments were carried out (three independent experiments for the doses 5 and 10 mGy), with at least 30 dechorionated embryos employed for each experiment. All embryos were dechorionated at 4 hpf. These embryos were then separated into two groups and accommodated in separated wells in a 6-well cell cultural dish with a layer of biocompatible agarose, referred to as:

Sham irradiated group (S): dechorionated embryos placed in the neutron irradiation room but without actual neutron irradiation;

Neutron irradiated group (I): dechorionated embryos exposed to the desired neutron dose at 5 hpf.

Fig. 1 shows the procedures for studying the neutron dose response on zebrafish embryos.

The zebrafish embryos were irradiated on the movable bed of NASBEE at 1835 mm (source target distance [STD]=1835 mm) downstream from the target. All irradiated embryos were placed within the uniform dose irradiation field with a diameter 26 cm ± 2%. The embryos were placed in a 6-well tissue culture dish with a layer of agarose gel at the bottom (1 mm thick) and 3 ml E3 medium (5 mM NaCl, 0.17 mM KCl, 0.33 mM $CaCl_2$, 0.33 mM $MgSO_4$, 0.1% methylene blue) in each well. After irradiation, a Geiger survey meter (TGS-133, Hitachi Aloka Medical, Ltd., Tokyo, Japan) was used to measure the ambient dose rate to confirm that both the medium and samples were not activated. After that, all embryos were returned to the incubator with the temperature kept at 28 °C until they developed into 25 hpf.

2.4. TUNEL assay

Apoptosis was chosen as the biological endpoint for the present study. To detect the amount of apoptotic cells within the embryos, terminal dUTP transferase-mediated nick end-labeling (TUNEL) assay was employed at 25 hpf. The feasibility of using the TUNEL assay for apoptosis to establish the dose response in the zebrafish embryos was successfully demonstrated by the pioneer work of Bladen et al. (2007). It is true that the number of apoptotic signals will vary with time. However, earlier time points were not feasible due to the high apoptotic activity in zebrafish embryos as a result of organogenesis processes (Chen and Cheng, 2003) while later time points were not feasible due to the pigment development on the zebrafish embryos (Bladen et al., 2005).

The 25 hpf embryos were first fixed in 4% paraformaldehyde (Wako Pure Chemical Industries. Ltd., Osaka, Japan) in phosphate buffered saline (PBS) (Mediatech Inc., A Corning Subsidiary, VA, USA) with 0.1% Tween 20 (Sigma-Aldrich, Inc., Missouri, USA) at room temperature for 5 h. After that, the fixed embryos were dehydrated, and were then rehydrated before treating with 20 μg/ml protease kinase (Wako Pure Chemical Industries Ltd., Osaka, Japan) for 10 min. After the protease kinase treatment, the embryos were fixed again in 4% paraformaldehyde in PBS with 0.1% Tween 20 for 2 h. An *in situ* apoptosis detection kit (MK500, Takara Bio. Inc., Singa, Japan) was employed to perform TUNEL staining. Briefly, the fixed embryos were immersed on ice in the permeabilization buffer for 30 min. The apoptotic cells were labeled by staining the embryos in the mixture of labeling safe buffer containing Fluorescein labled-2′-Deoxyuridine, 5′-Triphosphate, FITC-dUTP and Terminal Deoxynucleotidyl Transferase (TdT) enzyme in the ratio of 9–1. The embryos were then incubated in a 37 °C humidified chamber for 110 min. Finally, the stained embryos were washed thoroughly by PBS in 0.1% Tween 20 for five times. The apoptotic signals within the whole embryos were captured by a confocal laser microscope (FV-1000, Olympus Corporation, Tokyo, Japan) with 4× objective lens (NA:0.16, UPLSAPO 4×, Olympus Corporation, Tokyo, Japan). For each embryo, a total of 15–25 sliced images (2.12 × 2.12 mm^3, 2.06 μm/pixel) with 25 μm intervals were captured from top to bottom.

2.5. Data analysis

The number of apoptotic signals within each 25 hpf embryo after TUNEL assay was counted using the ImageJ software which was freely obtainable from the website http://rsb.info.nih.gov/ij/. The image obtained from the confocal microscope was firstly converted into a binary image. The number of apoptotic signals was then obtained by the "Analyze particle" function in ImageJ. *T*-tests were used to assess the statistical significance for differences between samples. A *p* value smaller than 0.05 was considered to correspond to a statistically significant difference.

3. Results

Apoptosis signals revealed by the TUNEL assay on the 25 hpf zebrafish embryos were chosen as the biological endpoint for the present study. Representative combined images of stained embryos captured by a confocal laser microscope after receiving different neutron doses, viz., 0, 1, 20, 50, 100 and 200 mGy, are shown in Fig. 2.

The effects of neutron doses in the range from 0.6 to 100 mGy were studied. A total of 10 different neutron doses (namely, 0.6, 1, 2.5, 5, 10, 20, 25, 50, 70 and 100 mGy) were employed. For each independent experiment, the mean number of apoptotic signals (N) for the ***S*** and ***I*** groups were denoted as N_S and N_I, respectively. When N_S was interpreted as the average background apoptotic signal for the embryos in the corresponding set of experiment, the "net normalized apoptotic signals" for all the ***I*** groups could be determined as $N_I{}^+ = [(N_I - N_S)/N_S]$.

The results are also plotted in Fig. 3. By using the data for neutron doses of 10, 20, 25, and 50 mGy, we obtained the best fit line as $N_I{}^+ = 0.5073(\pm 2.7165) + [0.006150(\pm 0.06608) \times \text{Neutron dose (mGy)}]$ using Origin 8 SRO v8.0725 (OriginLab Corporation, Northampton, MA, USA), with adjusted $R^2 = 0.7891$, where the values in the brackets represent the standard errors of the fitted parameters. The 95% confidence bands were also plotted. The data for neutron doses of 0.6, 1, 2.5 and 5 mGy were excluded because they had negative values of $N_I{}^+$ which likely represented the presence of hormetic effects induced by neutrons (together with the associated 14% contamination of gamma rays). The $N_I{}^+$ values for neutron doses of 70 and 100 mGy were significantly below the lower 95% confidence band of the best-fit relationship between $N_I{}^+$ and the neutron dose. As such, these two points strongly suggest the presence of gamma-ray hormesis. Using a similar idea

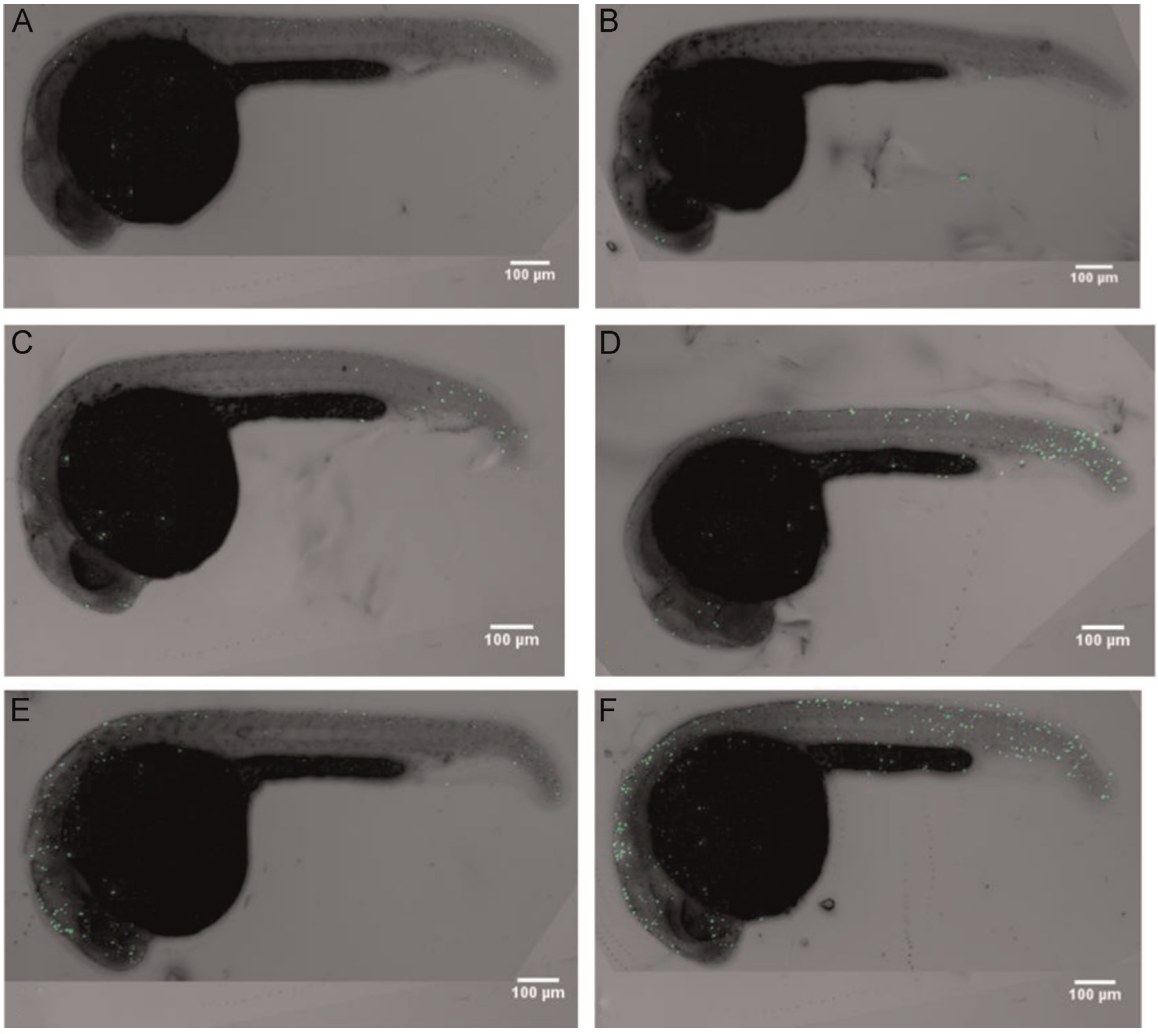

Fig. 2. Representative images of stained embryos after receiving different neutron doses, which were (A) 0 mGy, (B) 1 mGy, (C) 20 mGy, (D) 50 mGy, (E) 100 mGy and (F) 200 mGy, respectively. Images of embryos were captured by a confocal laser microscope with 4 × objective lens. A total of 15–25 sliced images with 25 μm intervals were captured for each embryo and combined from top to bottom.

underlying the definition of *PROFAC* by Rithidech and Scott (Rithidech and Scott, 2008), we here define $PROFAC^+$ as 1 – [(observed $N_I{}^+$ value)/(expected $N_I{}^+$ value)], and obtained $PROFAC^+$ as 58% and 54% for neutron doses of 70 and 100 mGy, respectively.

4. Discussion

In the present study, the basic protocols and techniques were developed for irradiation of zebrafish embryos with neutrons at the NASBEE facility in NIRS, Chiba, Japan. The low-dose neutron dose response of zebrafish embryos irradiated at 5 hpf was determined in the neutron-energy range from 0.6 to 100 mGy based on the number of apoptotic signals within the whole 25 hpf zebrafish embryos. We understood that gamma-ray hormesis was likely induced against alpha-particle irradiation when the gamma-ray doses reached 1–2 mGy (e.g., Refs. Scott 2008; Scott et al., 2008) and beyond. However, neutrons might inflict different DNA damages and thus initiate subsequent repair processes. For example, while alpha-particle induced RIBE appeared to be generic, RIBE did not seem to be induced by neutrons between cells *in vitro* (Liu et al., 2006; Seth et al., 2014) or between zebrafish *in vivo* (Wang et al., 2011). As such, the gamma-ray doses of 1–2 mGy for induction of gamma-ray hormesis against alpha-particle irradiation might not be applicable in the case of neutron irradiation. We focused on neutron doses ≤ 100 mGy because excessively long irradiation time (with little medium) might over-stress the zebrafish embryos and led to extra apoptotic signals. For example, the large $N_I{}^+$ value for an experiment with a neutron dose of 200 mGy shown in Figs. 2 and 3 might indicate the extra apoptotic signals due to over-stress. There were more measurements at smaller neutron doses in case there was a hormetic effect caused by neutrons (i.e., neutron hormesis).

From the present data, as gamma-ray hormesis appeared to be operative for neutron energies > 50 mGy, the negative $N_I{}^+$ values corresponding to neutron doses of 0.6, 1, 2.5 and 5 mGy likely represented the hormetic effect induced by neutrons (together with 14% contamination of gamma rays). The (neutron) hormetic zones and toxic zones appeared to be separated at some specific dose value somewhere between 5 and 10 mGy. The existence of hormesis in the low-dose neutron dose response relationship did not fit the linear-no-threshold (LNT) hypothesis. Hormetic zones and toxic zones were previously observed in zebrafish embryos irradiated at 1.5 hpf with α-particles (Yum et al., 2010) and in zebrafish embryos with both cells irradiated at the 2-cell stage with protons (Choi et al., 2012b). These results suggested that hormetic responses were generically developed by different kinds of ionizing radiations with different linear energy transfer (LET) values.

When the neutron dose was increased to above 10 mGy, the numbers of apoptotic signals developed on the irradiated embryos

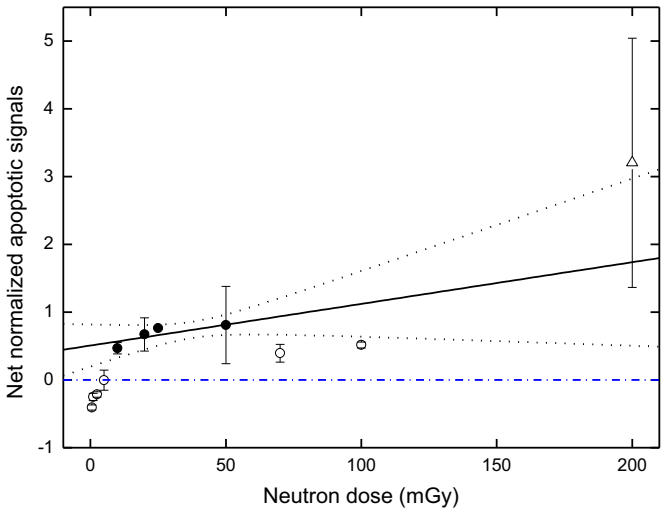

Fig. 3. The net normalized apoptotic signals (N_I^+) for 25 hpf zebrafish embryos which have been irradiated to different neutron doses at 5 hpf. *Solid circles*: data used in the linear fitting (for neutron doses of 10, 20, 25, and 50 mGy); *open circles and open triangle*: data not used in the linear fitting. All data represent the averages from at least 2 independent experiments, except that for 200 mGy (open triangle). Error bar for the data point for 200 mGy represents the standard deviation while error bars for all other data points represent the standard errors; *Dot-dashed line:* $N_I^+ = 0$; *Solid line:* best fitted line given by $N_I^+ = 0.5073(\pm 2.7165) + [0.006150(\pm 0.06608) \times$ Neutron dose (mGy)], with adjusted $R^2 = 0.7891$; *Dotted lines:* 95% confidence bands.

were all larger than those in the sham-irradiated embryos, and the differences were all statistically significant ($p < 0.05$). The toxicity of the neutron dose of 100 mGy on the neutron-irradiated adult zebrafish was also confirmed by Wang et al. (2011). Toxic effect was also generally observed for high-dose neutron irradiation, e.g., in the range from 0.2 to 3 Gy (Lee and Kim, 2007; Vanderisickel et al., 2010).

In the toxic zone, the net normalized apoptotic signals N_I^+ for neutron doses of 70 and 100 mGy were significantly below the lower 95% confidence band of the best-fit relationship between N_I^+ and the neutron dose obtained from all the data in the toxic zone except these two doses. As such, it was likely that these two points reflect the presence of gamma-ray hormesis. We obtained *PROFAC*$^+$ as 58% for the neutron dose of 70 mGy (with a gamma-ray dose of 9.8 mGy) and 54% for the neutron dose of 100 mGy (with a gamma-ray dose of 14 mGy). Apparently, the gamma-ray doses required to induce gamma-ray hormesis against neutron irradiation were much higher than those ($\geq$1–2 mGy) required against alpha-particle irradiation (Scott, 2008; Scott et al., 2008).

As described in the Introduction, Rithidech and Scott (2008) employed data from human lymphocytes irradiated with mono-energetic neutron sources with five different energies, namely, 0.22, 0.44, 1.5, 5.9 and 13.7 MeV, to determine the contribution of gamma-ray hormesis to low-dose neutron irradiations. The accompanied gamma-ray doses for these sources were approximately 1%, 1%, 2%, 6%, and 6%, respectively, of the total doses. For each neutron energy, three separate total absorbed doses were studied, namely, 10, 50 and 100 mGy. The authors revealed the gamma-ray hormesis for a particular group (e.g., for 1.5-MeV neutrons and total absorbed dose of 10 mGy) through the protection factor *PROFAC*, where the expected number of micronucleated cells was evaluated as the product of the total number of cells scored in the group with the fraction of micronucleated cells in the combined data for neutron energies of 0.22 and 0.44 MeV (see Table 5 in their paper). However, such calculations did not reveal the potential variation of the gamma-ray hormesis (surrogated by *PROFAC*) with the total absorbed dose for the same neutron energy, which was examined in the present work. In order to enable comparisons, we re-analyzed the data provided in Table 3 of Rithidech and Scott (2008) and gave the results in Table 1. For a particular neutron energy group, there were data for four different total doses, namely, 0, 10, 50 and 100 mGy. We treated the data for 0 mGy as baseline values, and then used the proportion of neutron-induced micro-nucleated cells (referred to as the MN rate) to compute the "net normalized MN rate" as [(MN rate for a particular dose)–(MN rate for 0 mGy)]/[MN rate for 0 mGy], which was in line with our definition of the "net normalized apoptotic signals", i.e., N_I^+. Table 1 shows the revised gamma-ray protection factors (*PROFAC'*) for suppressing the number of neutron-induced micro-nucleated cells, reflected through the lower "observed net normalized MN rate" when compared to the "expected net normalized MN rate". It was noted that the baseline MN rate of 0.01273 for the 0.22-MeV neutron group was significantly larger than those shown in other groups, viz., it was more than 2.35

Table 1
Revised gamma-ray protection factors (*PROFAC'*) for suppressing the number of neutron-induced micro-nucleated (MN) cells recalculated using the data from Rithidech and Scott (2008).

Neutron energy (MeV)	Total dose (mGy)	Total cells	MN cells	Observed MN rate	Observed net normalized MN rate	Expected net normalized MN rate	*PROFAC'*
0.22	0	12019	153	0.0127			
0.22	10	8882	150	0.0169	0.3267		
0.22	50	8658	185	0.0214	0.6785	1.6333	0.58
0.22	100	11744	386	0.0329	1.5820	3.2665	0.52
0.44	0	13680	74	0.0054			
0.44	10	12027	93	0.0077	0.4295		
0.44	50	11964	182	0.0152	1.8122	2.1474	0.16
0.44	100	9625	278	0.0289	4.3395	4.2949	–0.01
1.5	0	9,060	42	0.0046			
1.5	10	9769	60	0.0061	0.3249		
1.5	50	9827	99	0.0101	1.1732	1.6245	0.28
1.5	100	9873	162	0.0164	2.5395	3.2489	0.22
5.9	0	10,942	48	0.0044			
5.9	10	13730	99	0.0072	0.6437		
5.9	50	12787	139	0.0109	1.4780	3.2185	0.54
5.9	100	10526	162	0.0154	2.5084	6.4369	0.61
13.7	0	11,025	26	0.0024			
13.7	10	9833	39	0.0040	0.6818		
13.7	50	8150	40	0.0049	1.0812	3.4092	0.68
13.7	100	9997	93	0.0093	2.9447	6.8184	0.57

times the rate of 0.005409 for the 0.44-MeV neutron group. The underlying reasons were not yet clear so we only focused on the other four data sets for our discussion.

The *PROFAC*' values were very small for gamma-ray doses ≤ 2 mGy with the mean ($\pm$ standard deviation)$=0.16$ (± 0.13), which strongly suggested that gamma-ray hormesis was not operative. This confirmed that gamma-ray doses of 1–2 mGy for induction of gamma-ray hormesis against alpha-particle irradiation were not applicable to the case of neutron irradiation, which was also demonstrated in our results shown in Fig. 3. The *PROFAC*' values obtained in our experiments of 58% and 54% gamma-ray doses of 9.8 and 14 mGy, respectively, were in general commensurate with the trends (larger *PROFAC*' values for larger gamma-ray doses) shown in Table 1.

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