



Dosimetric study of radioadaptive response of zebrafish embryos using PADC-film substrates

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ABSTRACT

We found the alpha-particle priming dose which could effectively induce *in vivo* radioadaptive response in the embryos of the zebrafish, *Danio rerio*, to be 4.8–48 μ Gy, through the apoptotic signals scored in the embryos at 24 h post fertilization. Specially fabricated thin polyallyldiglycol carbonate (PADC) films were used as support substrates for the dechorionated embryos. During irradiation, alpha particles passed through the PADC films to hit the zebrafish embryos, and the alpha-particle tracks were revealed in the films through chemical etching. By determining the number of alpha-particle tracks that coincided with the effective areas of the embryos, the alpha-particle doses were found.

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1. Introduction

Radioadaptive response (RAR) refers to the biological response where an exposure of cells or animals to a low dose of radiation (priming dose) induces mechanisms that protect the cells or animals against the detrimental effects of a subsequent larger radiation exposure (challenging dose). The phenomenon was first reported by Olivieri et al. (1984). RAR has challenged the linear-no-threshold (LNT) model widely adopted for radiation protection considerations and thus the estimation of radiation risk (Oughton, 2006). For the LNT model, an increase in the dose will lead to an increase in the risk. However, with RAR, an increase in the dose (through the priming dose) will lead to a decrease in the risk (of the challenging dose), which contradicts the LNT model. Research works have demonstrated the existence of RAR *in vitro* (e.g. Olivieri et al. 1984; Ueno et al., 1996; Ikushima et al., 1996; Yatagai et al., 2008), and most of these studies used low linear energy transfer (LET) radiation for the priming and challenging exposure. Studies on the induction of RAR by high-LET radiations are relatively scarce (Gajendiran et al., 2001; Iyer and Lehnert, 2002). *In vivo* RAR was studied by a whole-body X-ray exposure of mice using chromosome aberration as the biological endpoint (Cai et al., 2003; Wang et al., 2004).

Induction of RAR depended on several factors, including the magnitude of the priming and challenging doses. Matsumoto et al.

(2007) reported that the priming dose range should be around 0.01–0.2 Gy using low-LET radiation, while Sorensen et al. (2002) and Sasaki et al. (2002) suggested the priming dose limit to be less than 0.1 Gy in mammalian cells. A priming dose range of 0.05–0.1 Gy for whole-body X-ray exposure on mice was employed by Yonezawa et al. (1996).

Our previous study on RAR used alpha particles for both priming and challenging exposures on embryos of the zebrafish, *Danio rerio*, as an *in vivo* model (Choi et al., 2010a). Studies on alpha-particle induced radiobiological effect are also relevant to the exposure to naturally occurring radon progeny which results in an internal alpha-particle irradiation (Yu et al., 1995, 1997, 1999, 2006). An important advantage of using zebrafish embryos was that the human and zebrafish genomes shared considerable homology, including conservation of most DNA repair-related genes (Barbazuk et al., 2000).

Choi et al. (2010a) showed that pre-exposure of zebrafish embryos at 5 h post fertilization (hpf) before a subsequent challenging alpha-particle exposure at 10 hpf could significantly reduce their numbers of apoptotic signals at 24 hpf. This suggested the induction of RAR on zebrafish embryos by using a 5 h time interval between the priming and challenging doses. Considering the presence of a priming dose range in *in vitro* studies, we hypothesize that there is a range of priming doses which can induce RAR in zebrafish embryos by alpha particles. The objective of the present work was to explore the alpha-particle radiation dose required as the priming dose to induce RAR.

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Our previously designed experimental setup to study RAR induced by alpha particles in zebrafish embryo (Choi et al. 2010a) was followed. Zebrafish embryos were dechorionated at 4 hpf and irradiated at 5 hpf (priming exposure) and 10 hpf (challenging exposure). RAR was quantified by the number of apoptotic signals in the embryos at 24 hpf.

Polyallyldiglycol carbonate (PADC) films were used to quantify the radiation dose. PADC film is a commonly used solid-state nuclear track detector (SSNTD) (Nikezic and Yu, 2004). PADC films were used as support substrates for the zebrafish embryos during alpha-particle irradiation, which would at the same time reveal the positions and incident energies of alpha-particle hits on the zebrafish-embryo cells.

2. Materials and methods

2.1. Preparation of PADC-film substrates

PADC film was commercially available with a thickness of 100 μm from Page Mouldings, Worcestershire. To enable alpha particles with an appropriate energy to reach the embryos, the PADC film should be sufficiently thin. The 100 μm PADC films were etched in 1 N NaOH/ethanol at 40 $^{\circ}\text{C}$ to achieve a thickness of 16 μm (Chan et al., 2007). A 16 μm PADC film was then glued by epoxy (Araldite[®] Rapid, England) to cover a hole with 3 mm diameter drilled on the bottom of a petri dish with a diameter of 60 mm to form our irradiation dish.

2.2. Irradiation of zebrafish embryos

Zebrafish embryos were dechorionated at 4 hpf. Embryos were divided into three groups, namely, the adaptive group (which received both priming and challenging doses), adaptive control group (which received only challenging doses) and dechorionated control group (which received no radiation dose) with around 10 embryos in each group. The dechorionated zebrafish embryos were placed on the irradiation dish (see Fig. 1). The priming exposure was delivered to the adaptive group of embryos at 5 hpf using a planar ^{241}Am source (with an alpha-particle energy 5.49 MeV under vacuum and an activity of 0.1151 μCi).

To find the absorbed dose delivered to individual embryos during the priming exposure, the positions of the embryos on the PADC film were captured by a microscope (Nikon) with a magnification of 20 \times before the priming exposure. The lens of the microscope was then interchanged with the ^{241}Am source without disturbing the positions and the orientation of the embryos. The determination of the absorbed dose will be described in Section 2.4. The duration of priming exposure was chosen as 6, 12, 24, 30, 36, 48 and 60 s.

After the priming exposure, each embryo in the adaptive group was marked with a number and placed separately into a 24-well multidish (Nunclon, Denmark). On the other hand, the other two groups of embryos were sham irradiated and placed separately into another 24-well multidish. The embryos were then incubated at

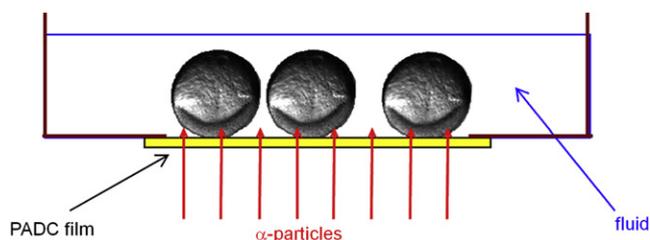


Fig. 1. Schematic diagram of our alpha-particle irradiation dish.

28.5 $^{\circ}\text{C}$ until 10 hpf. The adaptive group and adaptive control group of embryos at 10 hpf were irradiated for 240 s using the same ^{241}Am source as for the priming exposure. The embryos were returned back into the corresponding well after the challenging exposure.

2.3. Quantification of apoptotic signals

Apoptosis was the biological endpoint chosen in this study. At 24 hpf, the embryos were transferred into the culture medium containing 5 $\mu\text{g}/\text{ml}$ acridine orange. The embryos were stained for 60 min and washed twice in the culture medium. They were then anaesthetized by 0.016 M tricaine (Sigma, St. Louis, MO, USA). For each embryo, two to three images with focuses on different sections of the embryo were captured under a fluorescent microscope (Nikon) with a magnification of 40 \times . These images were then combined into a single embryo image for quantification of apoptotic signals. An apoptotic cell would appear as a bright dot in the image, and the number of apoptotic signals in each embryo was manually counted (Chan and Cheng, 2003; Yum et al., 2009, 2010).

2.4. Determination of the alpha-particle absorbed dose

To determine the dose absorbed by an embryo, the number of alpha particles (N_{α}) incident onto the effective area of the zebrafish embryo had to be revealed. Considering the energy loss of alpha particles when passing through the PADC film and the water column before reaching the embryos, the effective area was defined as the area in which alpha particles could reach the embryos (Yum et al., 2007).

The alpha-particle tracks were revealed by etching the PADC films in 6.25 M NaOH/aqueous at 70 $^{\circ}\text{C}$ for 3 h. Images of the enlarged alpha-particle tracks were captured by a microscope with a magnification of 200 \times . A total of about 45 images of the alpha-particle tracks were combined to reconstruct an overall image of the PADC film as shown in Fig. 2(a). The image of the embryos captured before priming exposure was superimposed onto the overall image of alpha-particle tracks using Ulead PhotoImpact Version 11.0 (Ulead Systems, Inc.) (see Fig. 2(b)). The effective areas of the embryos were plotted on the superimposed image using ImageJ (obtainable from the website <http://rsb.info.nih.gov/ij/>) to determine N_{α} . The alpha-particle radiation dose was calculated from N_{α} , the average energy of alpha particles located within the effective area, and the average mass of a zebrafish embryo at 5 hpf (Yum et al., 2007). The average mass was determined from the mass of 105 embryos obtained using an electronic balance (Sartorius, CP225D) after mild fixation under 3% glutaraldehyde for 18 min. The average mass of a 5-hpf embryo was measured as 255 μg .

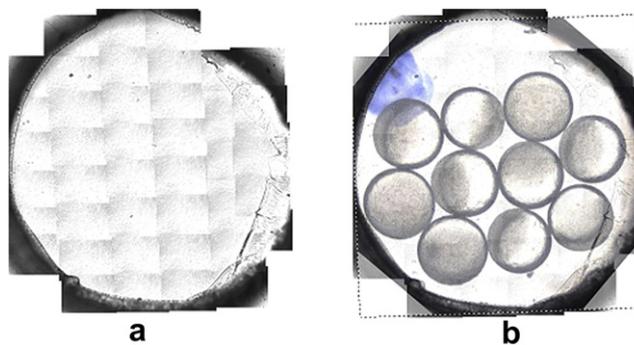


Fig. 2. (a) An image for the entire PADC film with visible tracks, which has been reconstructed by combining 45 images. (b) Superimposed image of the embryos and the visible tracks.

which was slightly larger than the average mass of a 1.5-hpf embryo measured as 222 μg (Yum et al., 2009).

3. Results

The presence of RAR was reflected by the number of apoptotic signals revealed in 24 hpf zebrafish embryos. We considered the number of apoptotic signals from the dechorionated control group of embryos in each set of experiment as the background signals; both the number of apoptotic signals in the adaptive group and adaptive control group were first subtracted by the background signals. The net apoptotic signals from the adaptive group were divided into six groups according to the number of alpha-particle hits on the embryos during the priming exposure (group 1 embryos had 1 to 10 alpha-particle hits, group 2 embryos had 11 to 20 alpha-particle hits, etc.).

Table 1 summarizes the mean values for the number of apoptotic signals for all groups. The mean values of different groups were compared with the mean values of the apoptotic signals from the adaptive control group (Group 0 as shown in Table 1) to calculate the difference D in apoptotic signals ($D = \text{mean of apoptotic signal for adaptive control group} - \text{mean of apoptotic signal for adaptive group}$). A positive D value suggested the presence of RAR. The significance between the differences was determined by the t -distribution after outliers, if any, were removed. A p value smaller than 0.05 was considered to imply a statistically significance for the difference. From Table 1, positive D values were obtained from Groups 1 to 4 with p values less than 0.05. The results suggested that RAR could only be induced when fewer than 40 alpha particles were incident onto the effective area of the embryos (i.e., or equivalently when the priming alpha-particle absorbed dose was less than 48 μGy). The most prominent adaptation (i.e., the largest value of D) occurred for Group 2 where the alpha-particle dose ranged from 13 to 24 μGy . The minimum number of alpha particles received by a zebrafish embryo during the priming exposure was 4, which corresponded to an alpha-particle absorbed dose of 4.8 μGy .

4. Discussion

The results showed a significant decrease of apoptotic signals after having a pre-exposure of alpha particles with a dose ranged from 4.8 to 48 μGy prior to a challenging dose of about 420 μGy . In fact, it was not straightforward to define the absorbed dose of alpha particles in the zebrafish embryos because the embryos had multiple layers of cells, and the alpha particles might only hit the outermost cells. The question remains whether only the mass of the cells actually hit by the alpha particles or the mass of the entire embryo should be employed in the calculation of the absorbed dose. Using the mass of the entire embryo might underestimate the

dose, while an accurate determination of the mass of a layer of cells presented a big challenge.

Two different methods were previously proposed by our group (Yum et al., 2007, 2009) to calculate the absorbed dose. The first method involved a theoretical calculation of the mass from the volume and density of the embryo cells by assuming a spherical shape for the embryo (Yum et al., 2007). The second method referred to a direct measurement of the average mass of the embryos using an electronic balance (Yum et al., 2009). As expected, the masses obtained by these two methods, and thus the calculated doses, were different. It would need more studies to be able to decide on the better choice or on possible alternatives. The absorbed dose calculated using the measured mass was found to be about 5 times lower than the one by calculating the mass of embryo cells. Without better information, we chose to use the measured mass of embryos in the present work.

Several previous studies reported on the ranges of priming dose for the induction of RAR *in vitro* (Matsumoto et al., 2007; Sorensen et al., 2002; Sasaki et al., 2002), viz., from 0.01 to 0.2 Gy for low-LET radiation. The priming dose range (4.8–48 μGy) obtained in this study was much smaller than those obtained in *in vitro* cases. The difference would remain significant even when our values were increased, say by 5 times, on using a different approach to determine the mass. The biological effectiveness between high-LET and low-LET radiations might further explain part of the difference. However, the validity of making comparison between the effective doses of different types of radiation at low doses by using radiation weighting factors W_R might have problems (Mitchel, 2007). If effective doses are compared, the priming effective dose capable of inducing RAR from alpha particles reported here (96–960 μSv) were comparable to that from protons (220–860 μSv) reported by Choi et al. (2010b). Incidentally, the two ranges overlapped with each other.

Most previous studies on the induction of RAR involved the use of low-LET radiation, in which all the cells were hit. Interestingly, RAR was also induced in the present work by 4–40 alpha particles. In this dose range, it was not possible for all the cells of the embryo to be hit, and it was also unlikely that the cells hit by alpha particles during the challenging exposure to be the same as those cells hit by alpha particles during the priming exposure. As such, it was likely that the RAR in the zebrafish embryos for low dose alpha-particle irradiation developed through the cell-to-cell bystander effect. The radiation-induced bystander effect (RIBE) was extensively studied in the past decades since its observation by Nagasawa and Little (1992). Growing evidences have demonstrated that irradiated cells might release stress signal factor(s) to affect the neighboring cells, or affect the cells which have received the medium conditioned by the irradiated cells (see for example reviews by Little, 2006; Mothersill and Seymour, 2006; Morgan and Sowa, 2007). Recently, our group also found that RAR could be induced in naive zebrafish embryos through RIBE (Choi et al., 2010c).

Table 1

Mean apoptotic signals ($\pm\text{SE}$) for adaptive group of embryos and their p values when compared with Group 0 (results of adaptive control group). $D = (\text{mean of apoptotic signal for adaptive control group} - \text{mean of apoptotic signal for adaptive group})$ ($\pm\text{SE}$); p : p values determined from t -distribution (cases with $p < 0.05$ are asterisked).

Group	Priming Dose (μGy)	Mean	D	p
0	0	77 \pm 5	0 \pm 8	/
1	4.8–12	40 \pm 9	35 \pm 12	0.00047*
2	13–24	31 \pm 5	44 \pm 10	3.6 \times 10 ⁻⁸ *
3	25–36	53 \pm 6	22 \pm 9	0.0028*
4	37–48	48 \pm 6	27 \pm 12	0.00042*
5	49–60	93 \pm 5	-18 \pm 21	0.020*
6	61–72	93 \pm 14	-18 \pm 16	0.15

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