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Attempts of Radiation Dose Measurement in the Teeth of Mice Living around the Nuclear Power Plant in Fukushima Using Electron Spin Resonance Spectroscopy

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Electron spin resonance (ESR) spectroscopy in combination with irradiated solid granulated sugars was first examined for use as a radiation dosimeter. The first derivative ESR spectrum obtained from X-irradiated sugars was doubly integrated to derive the actual signal intensity. The amount of free radicals produced in X-irradiated sugars was estimated by comparing with the intensities of the 1.1-diphenyl-2-picrylhydrazyl (DPPH) standard having one spin (radical) in its molecular structure. The linear relationship obtained between the amount of free radicals and the irradiation dose in the range of 33 - 2000 mGy confirmed the applicability of ESR spectroscopy as a dosimeter. Therefore, this method was further applied to measure radiation doses accumulated in the teeth of field mice living around the Fukushima Nuclear Power Plant to assess the impact of the Fukushima nuclear accident that occurred on March 11, 2011. Nineteen field mice were collected between November 15 and 17, 2013. ESR signals of the teeth (40 mg for each) of these mice were compared with those in Hokkaido (non-irradiated controls). However, because of large background ESR signals in both samples, no statistically significant difference was observed between the radiation levels in the teeth of mice collected from Fukushima and those from Hokkaido.

Key words: ESR, dosimetry, X-irradiation, granulated sugars, teeth of field mice

1. Introduction

The nuclear accident at the Fukushima Daiichi Power Plant of the Tokyo Electric Power Company occurred on March 11, 2011. Much attention has been paid to

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the radiation exposure in the people living around the nuclear power plant. As a biophysical method to measure radiation dose in the human body, ESR (electron spin resonance) spectroscopy of the enamel of human teeth is considered to be useful[1-3]. In the present study, we examined whether the combination of ESR spectroscopy and granulated sugars is useful to measure the exposure doses less than 100 mGy which is the threshold dose of the deterministic effects in human. Since the quantity of free radicals produced by irradiation doses less than 100 mGy is close to the ESR detection limit[4, 5], the ESR signals were accumulated by tracing a magnetic field 100 times to improve the S/N ratio. Each signal was doubly integrated for it to be converted into intensity for quantitative measurements of free radical production per 1 Gy irradiation. This improved method provided a sensitive detection system for doses below 100 mGy, and hence was applied to measure the radiation dose in the teeth of field mice living around the nuclear power plant in Fukushima.

2. Materials and Methods

2.1. Quantitative measurements of free radicals in granulated sugars induced by a relatively low dose of X-irradiation with ESR for the assurance of a sensitive assay method

A commercial product of granulated sugars (400 mg for each) contained in a quartz ESR tube of 5 mm diameter was irradiated with X-rays at doses of 0 – 2000 mGy with a dose rate of 1.0 Gy/min using a Shimadzu PANTAK HF-320 X-ray generator (200 kV and 20 mA). Immediately after irradiation, ESR signals were obtained by tracing 18.4 mT magnetic fields for 3.6 s and repeating this process 100 times using a JEOL JES RE1X spectrometer. ESR conditions were 1 mW of microwave power with 9.08-GHz field modulation of 0.4 mT amplitude. Each ESR spectrum was doubly integrated using the WIN-RAD computer software (Radical Research Company, ver. 1.20) to derive the actual signal intensity. 1.1-Diphenyl-2-picrylhydrazyl (DPPH) (Wako Chemical Company, Japan) powder that was mixed with 400 mg of glycine powder in a mortar and adjusted to the concentration of 5.0×10^{15} spins (radicals) was used as a standard. The ESR signal of DPPH was doubly integrated under the same conditions as above and the signal intensity thus obtained was used to estimate the number of free radicals in not only irradiated granulated sugars but also the teeth of field mice.

2.2. Quantitative measurements of free radicals in the teeth of field mice collected from Fukushima and Hokkaido using ESR

Large Japanese field mice (*Apodemus speciosus*) were collected from three areas around the nuclear power plant (Tanashio, Murohara and Akogi in Namie-town of Fukushima) between November 15 and 17, 2013 (Table 1). Both the upper and lower teeth of 19 mice were collected and stored in a desiccator before use. As a non-irradiated control, the teeth of field mice (*Apodemus speciosus ainu*) in Tsukigata-town of Hokkaido were used. The enamel was not extracted from the teeth because of the limit in the amounts of collected samples.

For ESR measurements, 40 mg of teeth from each

Table 1. Data base of large Japanese field mice (*Apodemus speciosus*) collected from three areas around the nuclear power plant (Tanashio, Murohara and Akogi in Namie-town, Fukushima)

No	Day of collection	Day of extraction	Collection place	Sex	Weight (g)	Length (mm)
1	Nov 15, 2013	Nov 15, 2013	Tanashio	M	33.1	158
2	Nov 15, 2013	Nov 15, 2013	Tanashio	F	28.0	198
3	Nov 15, 2013	Nov 15, 2013	Tanashio	F	36.4	200
4	Nov 15, 2013	Nov 15, 2013	Tanashio	M	26.0	194
5	Nov 15, 2013	Nov 15, 2013	Tanashio	M	19.9	175
6	Nov 15, 2013	Nov 15, 2013	Tanashio	F	19.8	173
7	Nov 15, 2013	Nov 15, 2013	Tanashio	M	19.2	182
8	Nov 15, 2013	Nov 15, 2013	Tanashio	M	25.4	181
9	Nov 15, 2013	Nov 15, 2013	Murohara	M	18.8	170
10	Nov 15, 2013	Nov 15, 2013	Akogi	F	28.3	202
11	Nov 15, 2013	Nov 15, 2013	Akogi	M	37.6	203
12	Nov 15, 2013	Nov 15, 2013	Akogi	F	31.0	214
13	Nov 15, 2013	Nov 15, 2013	Akogi	M	37.5	228
14	Nov 15, 2013	Nov 17, 2013	Murohara	M	36.9	211
15	Nov 15, 2013	Nov 17, 2013	Murohara	M	32.8	205
16	Nov 15, 2013	Nov 17, 2013	Murohara	F	32.6	204
17	Nov 17, 2013	Nov 17, 2013	Akogi	F	46.3	221
18	Nov 17, 2013	Nov 17, 2013	Akogi	M	23.6	182
19	Nov 17, 2013	Nov 17, 2013	Akogi	F	19.2	153

mouse was placed in quartz ESR tubes of 5 mm diameter. ESR spectrum of Mn^{++} was recorded together with that of teeth of mice for correction of both magnetic field and signal intensity. Each ESR spectrum was doubly integrated to convert the signal into intensity as described above. The resulting signal intensities were divided into three groups according to the collection areas and the average values of intensities in the three groups were calculated. The average values of ESR intensities obtained from the mice in Hokkaido were also calculated similarly.

2.3. Statistical analysis

The results were expressed as mean ± SD. Statistical analysis was performed using the one-way ANOVA.

3. Results

3.1. ESR data obtained from the DPPH standard

The first derivative ESR spectrum of DPPH corresponding to 5.0×10^{15} spins in 400 mg glycine powder is shown in Figure 1a. The integrated and doubly integrated ESR spectra are indicated by i and di, respectively, in Figure 1b. The height of the spectrum di corresponds to the signal intensity of 5.0×10^{15} radicals. This value was used to estimate the number of free radicals in the irradiated granulated sugars and the teeth of mice.

3.2. Dose-response curve of X-irradiated granulated sugars

The first derivative ESR spectra of granulated sugars exposed to X-rays at doses of 0 - 2,000 mGy are shown in Figure 2a. Each spectrum was doubly integrated to convert the signal intensity to calculate the number of free radicals by comparing the intensity with that

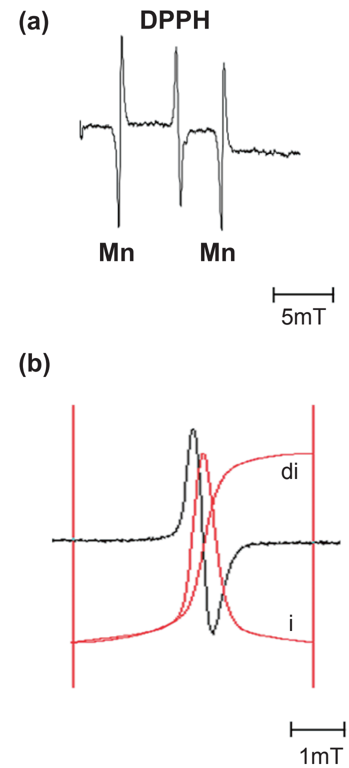

Fig. 1. (a) First derivative ESR spectrum of DPPH corresponding to 5.0×10^{15} spins in 400 mg glycine powder. Abscissa shows a magnetic field. (b) Integrated and doubly integrated ESR spectra of the first derivative indicated by i and di, respectively. The height of the spectrum di indicates the signal intensity.

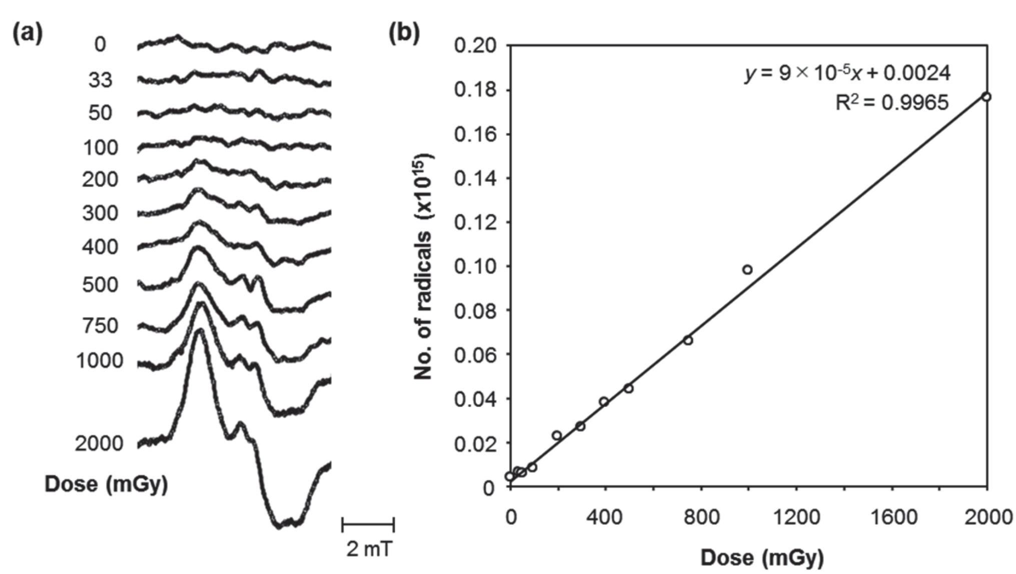

Fig. 2. (a) First derivative ESR spectra of granulated sugars exposed to X-rays of 0 - 2,000 mGy. (b) Number of free radicals induced by X-rays (Y-axis) vs. X-ray absorption dose represented by Gy (X-axis).

of DPPH. Figure 2b shows the dose-response curve of free radical production. X-axis and Y-axis show X-ray absorption dose (Gy), and the number of free radicals produced in granulated sugars, respectively. A linear relationship between the number of free radicals and irradiation dose (Gy) was observed. From the slope of the straight line, the number of free-radicals generated per 1 Gy irradiation of 1 g granulated sugars was calculated to be 2.1×10^{14}/g/Gy. This value corresponded to the generation rate of 1 radical per ~30 eV energy absorption (6.24×10^{15} eV/2.1×10^{14} radicals = 30 eV/radical). This almost accords with the W value (34 eV, the average energy of radiation to generate one ion pair). The minimum dose that was detectable by the ESR spectroscopy employed in this study was 33 mGy. When 400 mg of granulated sugars was exposed to X-rays with 33 mGy, the absolute number of free radicals generated was about 3×10^{12}. This value was considered to be the absolute spin number that was detectable by the present ESR technique.

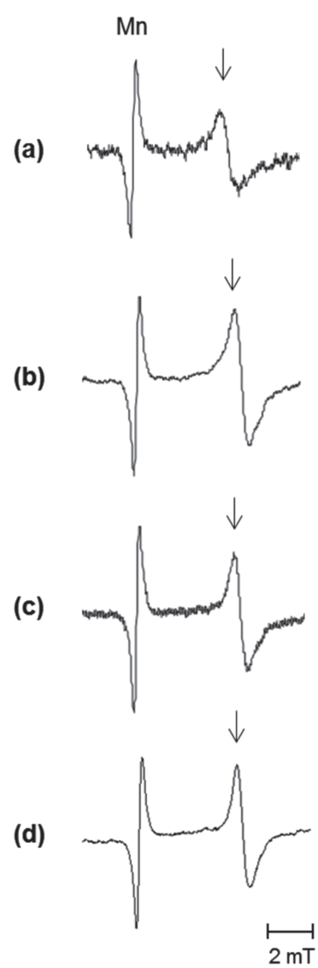

Fig. 3. The typical ESR spectra of the teeth of field mice collected from (a) Tsukigata, Hokkaido as a control, and (b) Tanashio, (c) Murohara and (d) Akogi, around the nuclear power plant in Namie-town, Fukushima. The 3rd peak of ESR spectrum of Mn^{++} is shown on the low magnetic field (g = 2.0337). Arrows indicate the magnetic field corresponding to g = 2.

3.3. ESR measurements of the teeth of field mice in Fukushima and Hokkaido

ESR measurements were performed for 19 samples collected from Fukushima (Table 1) as well as 7 samples collected from Tsukigata-town of Hokkaido. Figure 3 shows the typical ESR spectra of 40 mg of teeth of field mice collected from Tsukigata-town of Hokkaido (control sample) and three areas around the nuclear power plant (Tanashio, Murohara and Akogi in Namie-town of Fukushima). The spectra were obtained by 100 times of superposition. ESR spectrum obtained from mice in Hokkaido is shown in Figure 3a. ESR spectra obtained from mice in Tanashio, Murohashi and Akogi, are represented in Figures 3b, 3c and 3d, respectively. The 3rd peak of the ESR spectrum of Mn^{++} is shown on the low magnetic field (g = 2.0337) in each spectrum. Arrows indicate the magnetic field corresponding to g = 2. Following ESR measurements, all spectra were converted to the intensities by double integration. Each integrated value was corrected by the intensity of Mn^{++} spectrum simultaneously measured and changed to the relative intensity per 1 g sample weight. These values were distributed according to the area and the mean values were calculated and expressed as mean ± SD (Fig. 4a). The results were as follows; T (Tanashio) 0.12 ± 0.03, M (Murohara) 0.17 ± 0.07, A (Akogi) 0.14 ± 0.06, and C (control) 0.13 ± 0.03. No statistically significant difference was observed not only due to the high background intensity in the control sample but also due to the large variation among them. Figure 4b shows boxplots of these values in the four areas. Large variance and skewness can be observed in these values.

4. Discussion

The minimum dose that was detectable by the ESR spectrometer used in the present study was 33 mGy. As can be seen in the ESR spectrum of the control sample, a large spectrum was observed corresponding to the background signals equivalent to 2.1×10^{14} radicals that were approximately 70 times the number of free radicals obtained in granulated sugars irradiated with 33 mGy. No statistically significant difference was observed in the ESR signal intensities between the teeth of the non-irradiated control and those of the radiation-exposed mice in the three areas in Fukushima. This was due to the fact that most of these ESR spectra consisted of background signals. The radicals produced by ultraviolet exposure and unpaired electrons arising from lattice defects and impurities in the crystal of tooth are ESR detectable and considered to be responsible for background signals, but

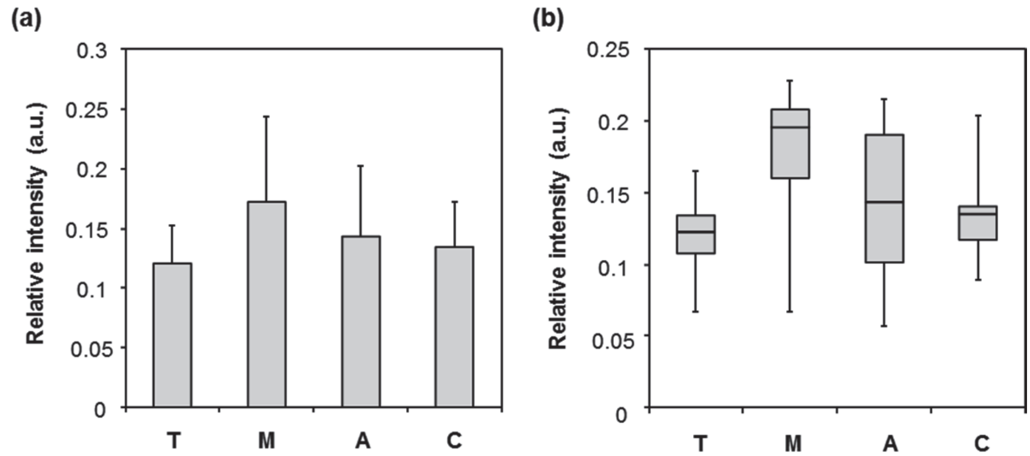

Fig. 4. (a) Bar graphs of ESR signal intensities at Tanashio (T, 8 samples), Murohara (M, 4 samples), Akogi (A, 7 samples), and Control (C, 7 samples). Each value is expressed as mean ± SD. (b) The boxplots of data obtained from the four areas. Maximum, 75%, median, 25% and minimum values are shown in each plot.

no details are well known.

It has been reported that γ-irradiated human teeth consisted of two main signals, CO_2^- radicals produced in the tooth enamel and organic radicals in the other parts[3]. When the teeth of mice were incidentally irradiated with 20 Gy of X-rays, an ESR spectrum originating from CO_2^- radicals produced in the tooth enamel was clearly observed in addition to that originating from the organic radicals (data not shown). However, ESR signals of CO_2^- radicals in all spectra shown in Figure 3 were too small to be separated. In the case of human teeth, tooth enamel was extracted and subjected to ESR measurements. In addition, the exposure doses were relatively high, and it was therefore possible to detect the ESR signals distinguishable from the background signals[1-3]. In the case of mouse teeth, whole tooth was used for ESR measurements because of the limit of its size and collected quantity. This was the reason why the radiation-induced signals were not distinguished from the background ones.

According to the Fukushima Prefecture radioactivity measurement map, the fixed-point measurements of spatial dose rates in three areas for 3 days from November 15 to 17, 2013 the mean spatial dose rates were as follows; 0.49 ± 0.1 (μGy/h) in Tanashio, 9.7 ± 1.5 (μGy/h) in Murohara and 14.8 ± 2.8 (μGy/h) in Akogi. The order of magnitude of these values was Akogi > Murohara > Tanashio. However, this order has not been reflected in the graphs in Figure 4. These values also indicate that the radiation dose rates were low. This fact suggests that the quantity of radicals is hard to be accumulated. However, it is well known that the radicals trapped in a crystalline materials are extremely long-lived[1, 3-5]. This means that the radicals are stably accumulated in teeth even by the low dose rate radiation. Based on these results, it was concluded that the measurement of radiation dose in the teeth of field mice living around the nuclear power plant was not possible by the ESR method. In addition, since the life of mouse is short (1-2 years), it may be difficult to use field mice in ESR dosimetry. As other biological materials for ESR dosimetry, human hair samples were used for relatively high dose measurement[6]. Authors concluded that these showed considerable variability and signal complexity creating difficulty in dose assessment.

Conflict of Interest Disclosure

The authors declare that they have no conflict of interest.

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