Chapter

Analysis of Radioactive Elements in Testes of Large Japanese Field Mice Using an Electron Probe Micro-Analyser after the Fukushima Accident

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Abstract

The Fukushima Daiichi nuclear power plant (FDNPP) accident drew global attention to the health risks of radiation exposure. The large Japanese field mice (*Apodemus speciosus*) are rodents endemic to, and distributed throughout, Japan. This wild rodent live in and around the ex-evacuation zone on the ground surface and/or underground. In this study, we evaluated the effect of chronic radiation exposure associated with FDNPP accident on the testes of large Japanese field mice. Morphological analysis and electron-prove X-ray microanalysis (EPMA) was undertaken on the testes. Morphological analysis of testes based on H&E staining showed that the spermatogenesis was observed normally in the breeding season of wild mice in the heavily contaminated area. However, caesium (Cs) was not detected in all testes of wild mice from FDNPP ex-evacuation zone. In conclusion, even if the testes and the process of spermatogenesis are hypersensitive to radiation, we could not detect radiation effects on the spermatogenesis and Cs in the examined large Japanese field mice testes following chronic radiation exposure associated with the FDNPP accident.

Keywords: EPMA analysis, Fukushima nuclear power plant accident, testis, wild mice

1. Introduction

The Fukushima Daiichi nuclear power plant (FDNPP) accident drew global attention to the health risks of radiation exposure. We have established an archive system composed of livestock and wild animals within a 20 km radius from FDNPP, that is, the ex-evacuation zone of the FDNPP accident [1–13]. This system provides critical information for the understanding of environmental pollution, biodistribution, radionuclide metabolism, dose evaluation, and the biological effects of internal and external exposure to radiation caused by nuclear disasters. In particular, experimental studies of low-dose rate (LDR) radiation exposure induced effects on spermatogenesis, along with indications from the nuclear disaster in Fukushima, will provide a more comprehensive radiobiological understanding of response mechanisms leading to improved accuracy in the estimation of human reproduction and health risk [14].

The large Japanese field mice (*Apodemus speciosus*) are rodents endemic to, and distributed throughout, Japan [15]. This wild rodent is appropriate for use as a reference animal of the ecosystem. Large Japanese field mice live in and around the ex-evacuation zone on the ground surface and/or underground. Hence, they are exposed to high levels of external radiation. Furthermore, they eat contaminated food and drink contaminated water. Consequently, they are directly affected by radioactive substances. Therefore, these mice can serve as a model to study the effect of radiation exposure, while also serving as a reference animal for the surrounding ecosystem.

Electron probe X-ray microanalysis (EPMA) is a powerful tool used to detect trace amounts of chemical elements in single cells and tissues [16]. This method measures the characteristic X-ray spectra of specific elements in samples using an accelerated electron beam. We previously investigated the effect of chronic LDR exposure to ^{134}Cs and ^{137}Cs on the testis of euthanised bulls, boars, and inobutas from the evacuation zone [3, 7].

Discharge of ^{134}Cs and ^{137}Cs that emit γ- and β-rays is of primary concern, because they were released in a large amount and have a long half-life. In this study, we evaluated the heavy contamination levels of LDR effects of ^{134}Cs and ^{137}Cs (between 4848 and 70,200 Bq/kg) on the large Japanese field mice after the FDNPP accident.

2. Materials and methods

2.1 Collections of large Japanese field mice

The study protocol followed laboratory animal care guidelines, and all procedures were conducted in accordance with the guideline of the Ethics Committee for Care and Use of Laboratory Animals for Research of Niigata University, Japan (approval number: H2611). Large Japanese field mice were captured using Sherman traps (H.B. Sherman Traps, Inc., Tallahassee, FL, USA) at three sites, Akogi, Ide, and Omaru of Namie town in the ex-evacuation zone of the FDNPP accident in November 2012, April 2013, and April 2016 (**Figure 1**). Control large Japanese mice were captured using Sherman traps in May 2012, November 2015, and April 2016 in Aomori Prefecture, and April and May 2016 in Niigata Prefecture. The ambient dose rate was measured at the sampling sites using NaI (Tl) scintillation survey meter TCS-171B (Hitachi Aloka Medical, Ltd., Tokyo, Japan) at the height of 1 m. The measurements were expressed as micrograys per hour at 1 m above the ground.

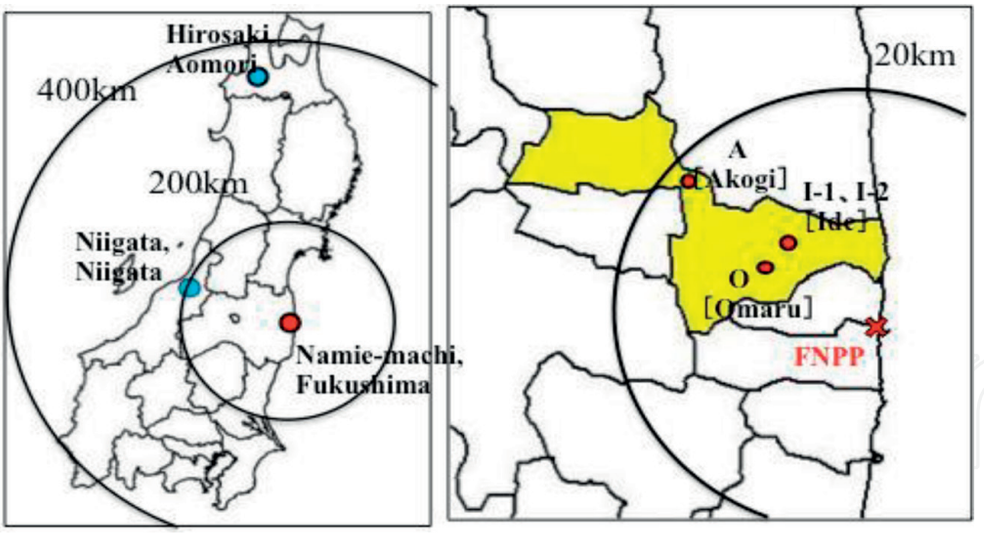

Figure 1.
Sampling site of in Namie town, Niigata and Aomori. Akogi, Ide and Omaru of Namie town in the ex-evacuation zone of the FDNPP accident is shown in yellow.

2.2 Measurement of radioactivity

Radioactivities of the organ samples were determined via gamma-ray spectrometry using high-purity germanium (HPGe) detector (GEM40P4-83, Ortec Co., Oak Ridge, TN, USA) as described previously [10]. The duration of the measurement varied from 110,600 to 663,400 s, depending on the radioactivity of the sample. Absolute efficiency of the detector was determined with the standard point sources of ^{137}Cs (10 kBq, CS402) and ^{152}Eu (10 kBq, EU402, Japan Isotope Association, Tokyo, Japan). The samples were placed in a small space (1 mm thick and 6 mm diameter) which is the same size as the standard point sources. A nuclide was identified when its characteristic photopeak 3σ above the baseline observed in the spectrum. The activities due to decay were corrected to the sampling dates.

2.3 Morphological assessment of testes cells

The testes were fixed in Bouin's solution, embedded in paraffin, and stained using haematoxylin and eosin (H&E), according to standard protocols, as described by Takino et al. [11]. Subsequently, the testes were briefly dehydrated in different concentrations of alcohol. The testes were made transparent by using toluene, and then, then they were embedded in paraffin and cut into 5 μm-thick sections before staining.

2.4 Electron probe X-ray microanalysis

Qualitative analysis: An analytical method was used to investigate the composition of the sample to be analysed. From ^{6}B to ^{92}U can be measured with a combination of analytical crystal to be used. The analysis conditions were as follows: voltage was set to 15 kV, beam current was 100 nA, beam size was minimum, sample current was 92.8 nA, and time 30 ms/point (**Figure 2**).

Elements analysis: Chemical trace analyses of caesium (Cs), sulphur (S), and nitrogen (N) in the testes were performed using a Shimadzu 1720HT electron probe micro-analyser (Shimadzu Corporation, Tokyo, Japan) equipped for X-ray spectrometry and specifically adapted for the examination of ultrathin sections. Accordingly, 3 μm of each testis section was placed on the carbon plate, and

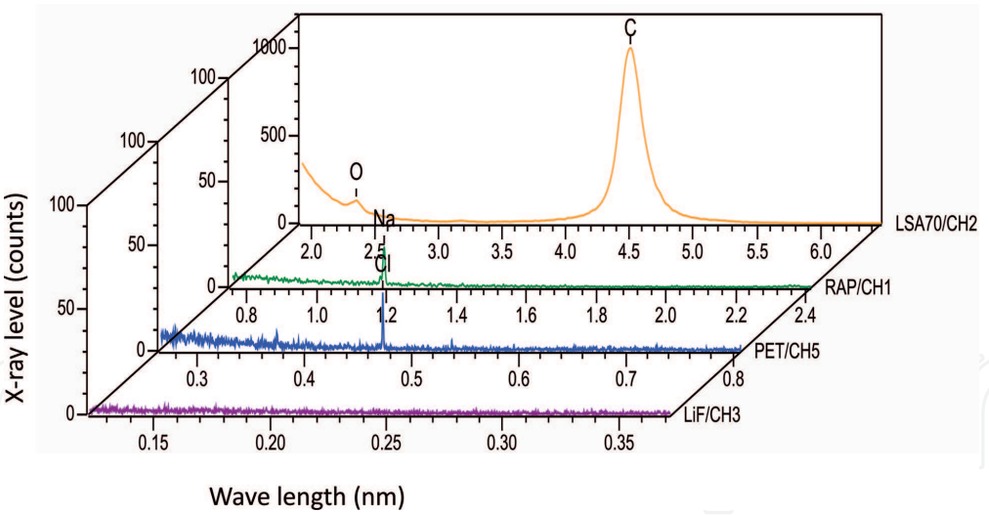

Figure 2.
Result of qualitative analysis by EPMA.

subsequently, each section was carbon coated for the electrification of samples (Biopathology Institute Co., Ltd., Oita, Japan). For the analysis, the voltage of the electron microscope was set to 15 kV, and the electron beam rate was set to 100 nA. Other parameters were beam size minimum × region (260 × 195 μm) and time (30 ms/point). The sections were viewed as secondary electron images, and chemical elemental mapping was performed. We performed EPMA analysis duplicate including test analysis.

3. Results and discussion

To date, low-dose radiation effects on physiological processes including spermatogenesis remain unclear. Further studies are required to confirm these low-dose radiation effects [14].

In the present study, we examined the effects of LDR exposure associated with the FDNPP accident on the testes of large Japanese field mice from different contaminated areas in the ex-evacuation zone, at Namie town in Fukushima. The ambient dose rate at Akogi was 26.9 μGy/h in November 2012, and 15.2 μGy/h in April 2013. The dose rate at Omaru was 12.3 μGy/h in April 2016. The dose rate at Ide was 16.4 μGy/h in April 2013, and 5.3 μGy/h in April 2016 (**Table 1**).

The ^{134}Cs and ^{137}Cs radioactivity concentrations (Bq/kg) in large Japanese field mice organ samples were detected via gamma-ray spectrometry by using an HPGe detector (**Table 1**). The total radioactivity concentrations of ^{134}Cs and ^{137}Cs in large Japanese field mice organ samples from Omaru were 2510, 2750, 3860, and 37,630 Bq/kg. Those from Ide were 10,820 and 16,550 Bq/kg, and this level is highly contaminated for the large Japanese field mice in the ex-evacuation zone.

Okano et al. [17] reported that, although the concentrations of ^{134}Cs and ^{137}Cs in wild mice from Fukushima exceeded 4000 Bq/kg, there were no significant differences in the frequencies of apoptotic cells or morphologically abnormal sperm when compared with wild mice from the non-contaminated control area. However, Kawagoshi et al. [18] reported that the average frequencies of chromosomal aberrations in splenic lymphocytes of animals living in the heavily contaminated (approximately 3 mGy/day) area of Fukushima were higher than those of animals from the non-contaminated, slightly contaminated (approximately 0.03 mGy/day), and moderately contaminated (approximately 1 mGy/day) areas. Moreover, the

Area	Large Japanese field mice			Sampling date	Ambient dose rate (μGy/h)	Body weight (g)	Radioactive concentration of ^{134}Cs and ^{137}Cs		
	No.	ID	Site				^{134}Cs (Bq/kg)	^{137}Cs (Bq/kg)	Total (Bq/kg)
Fukushima	1	215	Akogi	11/6/2012	26.9	23.5	–	–	–
	2	260		19/04/2013	15.2	32.4	–	–	–
	3	572	Omaru	12/4/2016	12.3	36.2	580	2880	3460
	4	575		12/4/2016	12.3	29.2	500	2250	2750
	5	590		12/4/2016	12.3	10.8	690	3170	3860
	6	594		12/4/2016	12.3	28.1	460	2050	2510
	7	595		12/4/2016	12.3	44.5	6360	31,270	37,630
	8	257	Ide	19/04/2013	16.4	30.2	–	–	–
	9	596		12/4/2016	5.3	50.1	1960	8860	10,820
	10	597		12/4/2016	5.3	43.5	2990	13,560	16,550
Aomori (control)	11	150	Hirosaki	29/05/2012	–	37	–	–	–
Niigata (control)	12	2721	Kakuta	20/11/2015	–	34.2	–	–	–
	13	2811		18/04/2016		43.8			

Table 1.
Individual information for large Japanese field mice.

aberration frequency in individual wild mice tended to increase with the estimated dose rates and accumulated doses. Takino et al. [11] reported that enhanced spermatogenesis occurred in large Japanese field mice living in and around the ex-evacuation zone of FDNPP. It remains to be elucidated whether the phenomenon, which is attributable to chronic LDR exposure, has a beneficial or adverse effect on large Japanese field mice.

Morphological analysis of testes based on H&E staining showed that the stages of the seasonal reproductive cycle were classified into reproductive, non-reproductive, and transition periods (**Figure 3A–D**; 1). During the reproductive seasons of the large Japanese field mice from Ide, spermatogonia, primary spermatocyte, secondary spermatocyte, and sperm were observed (**Figure 3B**). Interestingly, spermatogenesis was also observed normally in the breeding season of wild mice in the heavily contaminated area of Omaru (**Figure 3C**). Moreover, it was confirmed that the regression of sperm and seminiferous tubules during the non-breeding season of the wild mice in the most heavily contaminated area of Akogi were normally observed (**Figure 3D**).

Figure 3A–D (images 4–6) presents the phase maps obtained using the EPMA indicating micro-constituent concentrations namely, Cs, S and N. Colour imaging rapidly and effectively facilitates the overall analysis of the composite structure; specifically, decreasing levels of metal distribution are indicated from red to blue. Cs was not detected in all testes of wild mice from Ide, Akogi, and Omaru (**Figure 3A–D**: images 4). In the breeding samples, sulphur was detected inside seminiferous tubules, especially in sperm and was detected around the seminiferous tubules in the non-breeding seasons (**Figure 3A–D**: images 5). Nitrogen was detected inside both the seminiferous tubules and membranes (**Figure 3A–D**: images 6).

In conclusion, even if the testes and the process of spermatogenesis are hypersensitive to radiation, there were no significant radiation effects on the

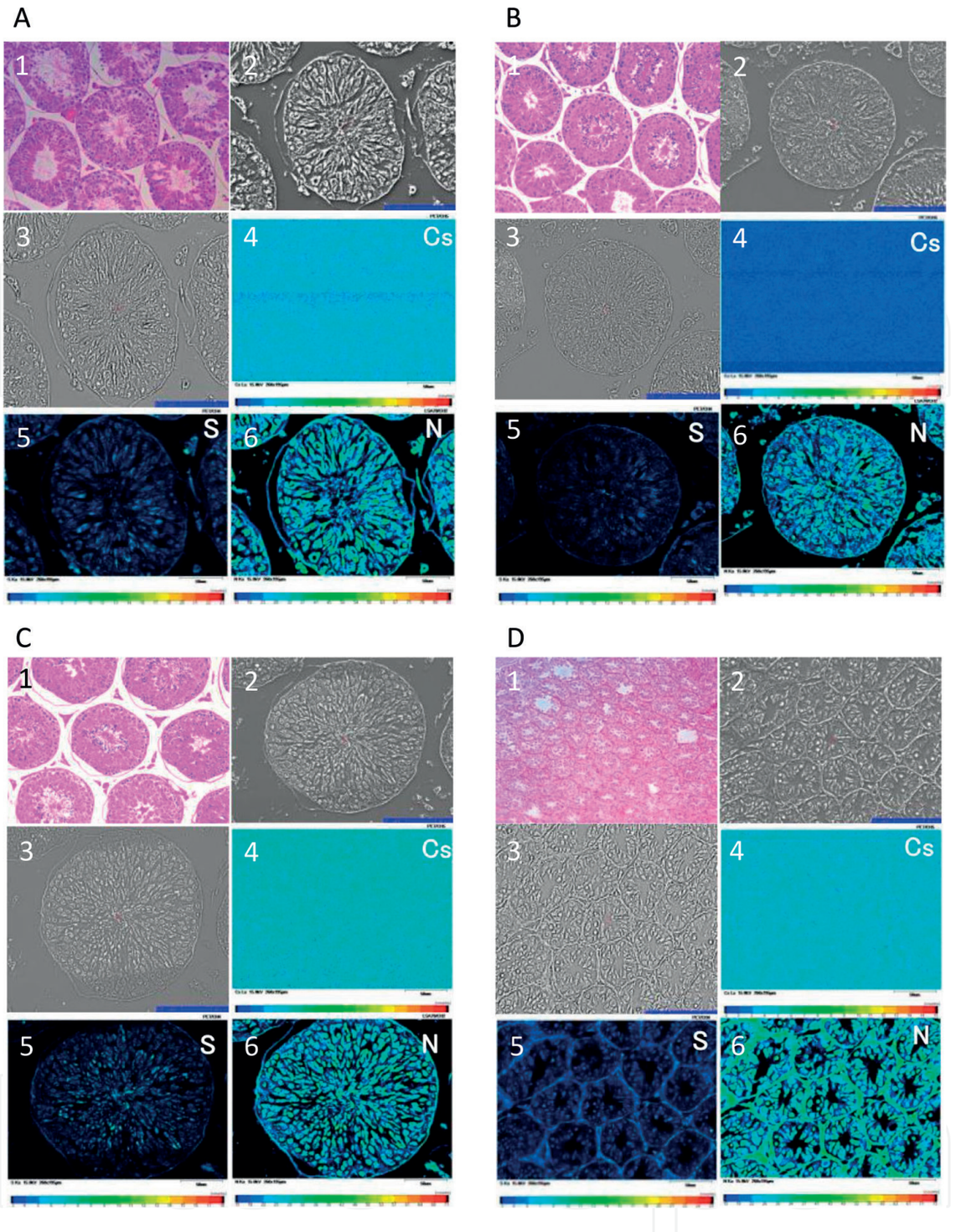

Figure 3.
Elements analysis of large Japanese field mice testis. A. Control (ID 2811), B. Ide (ID 596), C. Akogi (ID 215), and D. Omaru (ID 595). (1) H & E staining images of testis. (2) Stereo-microscopy images. (3) Composite backscattered microscopy images. (4) Colour map images of Cs (caesium). (5) Colour map images of S (sulphur). (6) Colour map images of N (nitrogen).

spermatogenesis and Cs in the examined large Japanese field mice testes following chronic LDR radiation exposure associated with the FDNPP accident.

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