A comprehensive dose evaluation project concerning animals affected by the Fukushima Daiichi Nuclear Power Plant accident: its set-up and progress

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ABSTRACT

It is not an exaggeration to say that, without nuclear accidents or the analysis of radiation therapy, there is no way in which we are able to quantify radiation effects on humans. Therefore, the livestock abandoned in the ex-evacuation zone and euthanized due to the Fukushima Daiichi Nuclear Power Plant (FNPP) accident are extremely valuable for analyzing the environmental pollution, its biodistribution, the metabolism of radionuclides, dose evaluation and the influence of internal exposure. We, therefore, sought to establish an archive system and to open it to researchers for increasing our understanding of radiation biology and improving protection against radiation. The sample bank of animals affected by the FNPP accident consists of frozen tissue samples, formalin-fixed paraffin-embedded specimens, dose of radionuclides deposited, etc., with individual sampling data.

KEYWORDS: Fukushima Daiichi Nuclear Power Plant, sample bank, archives, radiation effects, animals

INTRODUCTION

Establishment of a sample bank of animals affected by the FNPP accident

Explosions at the Fukushima Daiichi Nuclear Power Plant (FNPP) occurred after the Great East Japan disaster, and they released a huge amount of artificial radioactive substances into the environment; most of them were dispersed on 15 March 2011. Since then, people globally have been concerned about the late effects of radiation decades after the accident; even the acute effects were not detected. The evacuation zone was defined on 22 April 2011 as within a 20-km radius of the FNPP, and since then no one has been allowed to enter the area without permission from the Anti-Disaster Headquarters. Since April 2012, rearrangements of the restricted areas, including the evacuation zone, have been performed several times. We, therefore, term the area within a 20-km radius of the FNPP the ex-evacuation zone. On 12 May 2011, the Prime Minister ordered the Governor of Fukushima to euthanize livestock within the evacuation zone, preventing people from eating meats contaminated with radionuclides. With the intention of increasing understanding of the biological and human effects of both internal and external radiation exposure and of the spatio-temporal changes occurring after radioactive contamination, the aim of this project was to establish an archive system, to accurately evaluate radiation doses to individual

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organs from euthanized animals and to the environment at the contami-
nated site, and to preserve sample materials with data for future genera-
tions. There is no doubt that the technology associated with radiation
measurement and analysis of biological effects will develop further in the
future. This archive will become crucial to our understanding of the
effects of radiation on humans and on the earth.

MATERIALS AND METHODS
Sampling methods and establishment of a tissue bank
We organized a research group entitled the ‘Group for Comprehen-
sive Dose Evaluation in Animals from the Area Affected by the
FNPP Accident’ and started the research activity after overcoming
many difficulties and obstacles. We had to obtain finances for the
project and also obtain permission to enter the ex-evacuation zone
and to bring out organ samples (Fig. 1). Since 29 August 2011, we
have been performing sampling of organs and peripheral blood
from animals left behind in and around the ex-evacuation zone
of the FNPP accident. Euthanasia and sampling began from the live-
stock about which consent was obtained from dairy farmers. After
euthanasia was performed (by veterinarians from the Fukushima
prefecture), peripheral blood was obtained from the jugular vein.

Carcasses were carried by dump trucks to the burying place and put
into the trench dug for burying them. Dissection was carried out in
the trench, and the organs were divided into four groups: one for
the measurement of radioactivity, one for histological examination
and two for molecular study (Fig. 2). For molecular analysis, duplica-
tes of frozen tissues were stored in two different laboratories
(where electricity was supplied by two independent power compa-
ries) in case of power failure. We also separated the plasma from the
blood cells on the dissection site. All samples were collected and
divided according to the purpose of analysis (Fig. 3).

Sample control via a barcode system
All the materials were itemized and linked to their information via a
barcode issued for each individual animal (Fig. 4). Each barcode
was printed on a piece of tape by a PT-9800PCN desktop network
thermal label printer (Brother Industries, Nagoya, Japan), using its
attached software, P-touch editor. The barcode label was laminated
and was put into a plastic freezer bag with the registered organ tissue
samples associated with the particular animal. All sample data were
compiled and organized into a chart on a Microsoft Excel spreadsheet
controlled by a barcode system (Fig. 5).

Fig. 1. Comprehensive dose evaluation of animals within and around the ex-evacuation zone of the FNPP accident. This project
cannot be accomplished without the development of science, the understanding of people, the opening of information, and
adequate funding. A strong research group entitled the ‘Group for Comprehensive Dose Evaluation in Animals from the Area
Affected by the FNPP Accident’ (composed of five faculties of Tohoku University, six other universities, two national institutes
and a company specialized in chromosome analysis) has been established.
Fig. 2. Sampling activity. A rent-a-car of van has been maintained since the beginning of this project and equipped with devices for dissection and sampling, including a portable freezer, a centrifuge, electric saws and a power generator (A). Sampling of cattle was started at Kawauchi village (where informed consent from farmers was first obtained) and ended at Okuma town. The roads in the mountainous area were disrupted by the earthquake, so the route taken was dependent on the sampling location.
RESULTS AND DISCUSSION

As at the end of March 2015, we have collected samples from 302 cattle, 57 pigs, 200 Japanese macaque, 8 wild pigs and 5 horses (Fig. 6). We have identified radioactive substances and measured the radioactivity concentration in the various organs [1] and its effect on the testis [2]. Four years after the FNPP accident, we could detect 134Cesium (134Cs) and 137Cesium (137Cs) in all organs from all of the animals examined. We evaluated the effects of radiation by performing biochemical analysis of the plasma components and looking for pathological changes, and measured the radioactivity concentration in the organs from cattle obtained between August 2011 and August 2012. No significant abnormal data were found in the routine biochemical analysis. However, the cattle were suggested to be under slight stress (Urushihara et al., manuscript in prep.).

The number of Japanese macaque is annually controlled at city- and village-base as a group of harmful animals. We have, therefore, collected organs from euthanized Japanese macaque. Four years after the FNPP accident, only 134Cs and 137Cs were detectable on γ-spectrometry. It is reported that pancytopenia proportional to the radio-cesium concentration in the muscle was observed in the young monkeys of Fukushima city [3]. We have been collecting monkeys from Minamisoma city and Namie town, where the radio-contamination is much higher than in Fukushima city. However, we could not observe any significant association between organ cesium radioactivity and blood cell counts (manuscript in prep.). Thyroid cancer is thought to be the only cancer that is known to have been induced by the Chernobyl accident. The number of child thyroid cancer cases began to increase 5 years after the Chernobyl accident [4]. The amount of radionuclides released by the Chernobyl accident is estimated to be 5300 PBq; the amount released by the FNPP accident is 520 PBq, one-tenth that of the Chernobyl accident. Furthermore, >80% of the radionuclides released went into the Pacific Ocean [5]. Assuming that the biological effect of radiation is related to the exposure dose, the effect of the FNPP accident on human disease, if it occurs, will not be apparent until more than 5 years after the accident. Compared with the Chernobyl accident, relatively smaller amount of the radionuclides have been deposited in the ex-evacuation zone. In the light of these facts, a long-term and vigilant study of the various animals in the ex-

(B). The unleashed cattle were trapped by food and water. After each animal was identified from its ear tag, it was anesthetized deeply and euthanized, using a muscle relaxant. Peripheral blood was taken from the jugular vein (C). While the veterinarians were performing the euthanasia, we collected grass with bate, and soil (D). The animal carcasses were brought to a ditch dug on public land (E), where we performed dissection (F). At this location, the organs were separated into four groups; one group destined for radioactivity measurement, two for cryopreservation in the various laboratories, and one for paraffin-embedded blocks (G). Cesium is believed to share transport pathways with potassium (such as the energy-dependent pump and channel). We therefore separated the blood cells from the plasma to avoid diffusion from one to the other during the time between sampling and radioactivity measurement (H).

| Sampling                                  | Handling conditions | Purpose and analysis                           |
|-------------------------------------------|--------------------|-----------------------------------------------|
| Blood collection                          |                    |                                               |
| RNA extraction tube x 2                  | room temp.         | RNA extraction for gene expression analysis  |
| 15-ml tube (heparin sodium) x 2           | cool               | chromosome analysis                           |
| 15-ml tube (EDTA) x 2                    | frozen             | H2AX staining                                 |
| 50-ml tube (heparin sodium) x 2          |                    | DNA extraction for mutation analysis          |
| ctf (3000 rpm x 15 min)                  | 50 ml x 2          | radioactivity measurement                    |
|                                           | 3 ml (in 15-ml tube) x 2 | biochemical analysis                           |
|                                           | Buffy coat         | cryopreservation for biochemical analysis     |
|                                           | 15-ml tube         | cryopreservation for gene analysis            |
|                                           |                    | radioactivity measurement                    |
|                                           |                    |                                               |
| Organs                                    |                    |                                               |
| - thyroid, submandibular gland           |                    | pathological study                             |
| - gall bladder                           |                    | cryopreservation                               |
| - rib, eye ball, intestine (if obtained)  |                    | radioactivity measurement                    |
| - liver, spleen, kidney, round, skin      |                    | pathological and proteomics study             |
| testes                                    |                    |                                               |
| aspiration of cumulus-oocyte complexes   | 10% formalin       | in vitro fertilization and artificial         |
| maturate in vitro                        |                    | insemination for transgenerational study      |
| - bile, feces, urine                     |                    | cryopreservation                               |
|                                          |                    | radioactivity measurement                    |

Fig. 3. Handling and analysis of materials. Materials were divided up according to their intended analytical purpose.
Fig. 4. Sample control. All samples were itemized and issued with individual barcodes.

| No | Control No. | ID  | Sample No. | Individual ID No. | Remark         | Other ID1 | Other ID2 | Other ID3 | Sample data |
|----|-------------|-----|------------|-------------------|----------------|-----------|-----------|-----------|-------------|
|    | CA001-B001  | CA001 B001 | 0836509647 |                   | 964 | 2011.8.29 (1st) | f | Jpn Black |
|    | CA001-B002  | CA001 B002 | 0836509647 |                   | 964 | 2011.8.29 (1st) | f | Jpn Black |
|    | CA001-B003  | CA001 B003 | 0836509647 |                   | 964 | 2011.8.29 (1st) | f | Jpn Black |
|    | CA001-B004  | CA001 B004 | 0836509647 |                   | 964 | 2011.8.29 (1st) | f | Jpn Black |

Fig. 5. Connecting each sample and datum. A simple database was created using Excel (A). An information searching system was set up in conjunction with barcodes (B). Frozen tissue samples from each animal were stored in a single large freezer bag, which was labeled with the laminated barcode issued for that animal; tissue samples from the various organs were individually stored in small bags within the large bag (C).
evacuation zone will be necessary in order to understand the late effects of radioactive cesium on the ecosystem and on humans. Detailed data are available upon request. Please contact the corresponding author (MF).

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Fig. 6. The cumulative number of samples. The project commenced on 29 August 2011 and is continuing. In total, we obtained samples from 302 cattle and 57 pigs (finished on 31 March 2013) and 200 Japanese macaques.