Development of Dark-striped Field Mice, *Apodemus agrarius*, as a Biological Dosimeter in a Radio-ecological Monitoring System 3. Radio-sensitivity between *A. agrarius* and ICR Mice

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Abstract

This study examined the possibility of using striped field mice as a biological dosimeter or indicator for the environmental radio-surveillance. For this study, the external morphological characteristics and isoenzymic types of dark-striped field mice were studied after they were captured. Among the morphological external characteristics, the dark-brown coat, dark back stripe, head-to-tail length, tail length, and ear length matched the taxonomical characteristics of dark-striped field mice. The analyses on L-lactate dehydrogenase, aspartate aminotransferase, and malate dehydrogenese revealed that one species of dark-striped field mice, called *Apodemus agrarius*, was inhabitated throughout a wide range of Korea. On the other hand, *A. agrarius* and ICR mice to analyze their survival rate and frequency of micronuclei in peripheral polychromatic erythrocytes after irradiation (0, 0.5, 1, 3, 5, 7, 9 Gy). The $LD_{50/30}$ of *A. agrarius* and ICR mice were approximately 5 Gy and 7Gy, respectively. The results of the study reveal that wild *A. agrarius* have a high potential as a biological monitoring system to determine the impact of radiation effect in areas such as those within the vicinity of nuclear power plants.

Key words: *Apodemus agrarius*, radio-sensitivity, ecological and Biological dosimeter, and ICR.

Introduction

Regarding the management of nuclear power plants and the installation of facilities for radiation waste storage: social concerns over radiation safety are increasing. To understand how environmental radiation affects on human beings, the development of an unmanned monitoring system is required. The existing radio-environmental surveillance systems can be classified into physical and biological monitoring systems. The physical monitoring system includes food [1], assessment of radiation concentration in soil and water [2], the assessment of ambient dose rate, and the assessment of radioactive materials accumulated in trees and other plants [3]. On the other hand, wild small animals were reported to be effective biological indicators of environmental radiation after the Chernobyl incident [4,5,6]. Moreover, livestocks such as cows [7] also used as indicators for the biological effects of radiation, but the development of new models is required, as they involve many confounding factors. This study investigated the possibility of using dark-striped field mice as a biological dosimeter to assess the radiation effects on the enviro-ecology. The taxonomically classified dark-striped field mice, *A. agrarius*, were then irradiated to investigate their radio-sensitivity based on survival rates, body weight and micronuclei frequency in polychromatic erythrocytes. The radiation sensitivity betwen A.

agrarius and ICR mice was compared in this study.

Materials and Methods

Collection of dark-striped field mice and breeding: For this study, dark-striped field mice, sampled from five regions in Korea (Kyonggi, Chungchong, Cholla, Kyongsang and Kangwon province), were collected with Sherman trap (H.B. Sherman trap, USA) in a single month (October). The dark-striped field mice were subjected to controlled breeding by allowing the specimens to mate with only one partner at a time. Breeding was continually induced after the newborn mice were five weeks old by mating four females with one male. Female mice that were 5-6 weeks old were used for this study.

Analysis of morphological external characters: In order to classify the species of dark-striped field mice, the *Apodemus* taxonomic classification of Corbet [8] was used to examine the coat color and to measure the length between the head and the basal parts of the tail, tail length, and the length of the ears.

Analysis of isoenzymes: After assessing the morphological external characteristics, the livers of the mice with dark-colored stripes on their backs were collected and analyzed for the electrophoretypes of enzymes (Lactate dehydrogenase, aspartate aminotransferase, malate dehydrogenase) [9]. The comparison model for this study was the livers of an ICR mouse at 6 weeks age (female), and all processes were conducted under a temperature of 4°C.

Irradiation: In order to examine the radiation sensitivity of *A. agrarius* and ICR mice, the five groups with different doses (0, 0.5, 1, 3, 5, 7, 9 Gy) were irradiated with gamma rays (^{137}Cs, dose rate: 0.8 Gy/min).

Survival rate: After irradiation, the mice were kept in three sterilized cages in groups of five to examine their survival rate for 30 days. The chow (Samyang, Korea) and beading (JRS, Germany) were used with those exposed to gamma rays. The mice were given feed and acidified (pH 2.5) water freely. For this study, the survival rate of the mice for 30 days from irradiation was examined and it was determined that the dose ($LD_{50/30}$) eventually killed about half of the mice. As the birth rate of wild mice is low, the number of mice was restricted to fifteen per dose in this study.

Weight: The exposed *A. agrarius* and ICR mice were examined for 30 days to assess the changes in their weight.

Micronuclei in polychromatic erythrocytes: For the analysis of micronuclei in polychromatic erythrocytes of *A. agrarius* and ICR mice after irradiation, acridine orange staining methode was used Hayashi et al [10].

Statistical analysis: The survival rate after irradiation was analyzed using GraphPad Prism (Ver. 4). The micronuclei frequency in polychromatic erythrocytes for different doses of radiation was analyzed using SAS (Version 8.1).

Results

Survival rate: This study examined the survival rate of *A. agrarius* after irradiation. To our surprise, most of those exposed to 3 Gy to 9 Gy of radiation died within 10 days. Although this study could only use fifteen mice for each group, as field mice with low reproductive rates were difficult to find, the dose that killed 50% of the mice within 30 days was 5 Gy (Fig. 1). However, the survival rate of ICR mice was decreased from 5Gy after irradiation and $LD_{50/30}$ was 7.9 Gy

(Fig. 2).

Body weight: Examining the weight of dark-striped field mice after irradiation revealed that the weight of the 2 Gy and 3 Gy groups showed a slight increase compared with that of the 0.5 Gy group, but there was no significant difference. However, the groups higher than 5 Gy showed a drastic decrease in weight and died. However, the body weight of ICR mice were decreased highly after 9Gy irradiation compared with other irradiated groups.

Micronuclei in polychromatic erythrocytes: The micronuclei in polychromatic erythrocytes of *A. agrarius* were detected to understand the chromosomal damage after irradiation. The frequency of micronuclei was highest in those exposed to 3 Gy, but was impossible to examine in higher doses. However, the micronuclei frequency in polychromatic erythrocytes of ICR mice was increased with doses on day 3rd after irradiation.

Discussion

There have been reports on the development and usage of biological dosimeter for the surveillance of the biological effect of radiation emitted from nuclear power plants or related facilities. However, as the existing biological assessment dosimeter have many confounding factors, it was difficult to assess the radiation effect. For example, the *Clethrionomys glarelus, Microtus agrestis* and *A. sylvaticus* that were reported after the Chernobyl incident were not highly applicable as they only inhabited in a limited range of habitat and in low numbers [4,5]. Moreover, it is difficult to develop a cellular-genetic methodology to adequately assess the effect of radiation and identify the dose-response relationship. Recently, Kim et al reported that the relative radiation sensitivity of cows, sheep, pigs, and rabbits are 0.86, 0.98, 0.41, and 0.39, compared to that of humans, to reveal the importance of confounding factors in selecting a biological animal dosimeter[7]. This study investigates the possibility of using dark-striped field mice as the radiation bio-monitoring model by solving the weaknesses of the biological dosimeter mentioned above.

This study investigated the radiation sensitivity of *A. agrarius*. First, the *A. agrarius* clear dark-stripes on their coats are irradiated to determine which dose would kill 50% of the mice within 30 days. The study revealed that the $LD_{50/30}$ of *A. agrarius* was about 5 Gy and showing higher radiosensitivity than those of ICR (7.9 Gy). The results suggests that genetic condition is one of the major factors influencing radiation sensitivity in mice. Moreover, this value is similar to 3~5 Gy, the $LD_{50/60}$ value of humans, as reported by Fujita et al [11]. Based on the report of Kim et al [7] that compared the radiation sensitivity of mice and human and the report of Abramsson-Zetterberg et al [5] proposing that the radiation sensitivity of *A. flavicollis* and *A. sylvaticusis* is similar to that of CBA mice, the possibility of using *A. agrarius* as an environmental surveillance dosimeter can be assumed. Since one of the main purposes of this study is to interpret the effect of radiation emitted from nuclear power plants or facilities for radiation waste storage by using *A. agrarius,* this result is significant as basic data. In other words, the data obtained from this study on *A. agrarius* can be directly applied to humans.

When examining the biological effects of radiation on mice, chromosomal aberrations or micronuclei in bone marrow or spleen cells are generally examined. This study irradiated *A. agrarius,* stained the red blood cells from a peripheral blood with acridine orange, and analyzed the micronuclei frequency in polychromatic erythrocytes. The 3 Gy groups exhibited the highest number of micronuclei, but it was impossible to examine the micronuclei in other higher doses. This reveals that the radiation sensitivity of *A. agrarius* makes their marrow cells vulnerable to irreversible damage when exposed to radiation doses exceeding 3 Gy. This is one of the reasons why the death rate of the mice increased drastically after five days from 3 Gy after irradiation. In this study, the *A. agrarius* radiated with 1 Gy and 3 Gy showed an increase in weight compared to those radiated in 0.5 Gy. This is a pathological phenomenon after irradiation, and these results are similar to those reported by Tsiperson and Soloview [6], who examined the increase in the weights of bodies and organs of bank voles and shrews due to chronic exposure (0.07 Gy/year) around Chernobyl. The data accumulated by studying *A. agrarius* can be applied to human beings, because the $LD_{50/30}$ was revealed to be approximately 5 Gy and cellular-genetic responses against radiation dose were identified. However, a study on the dose-response relationship and its correlation to human response against low-dose or low-dose-rate radiation is required deeply in the future.

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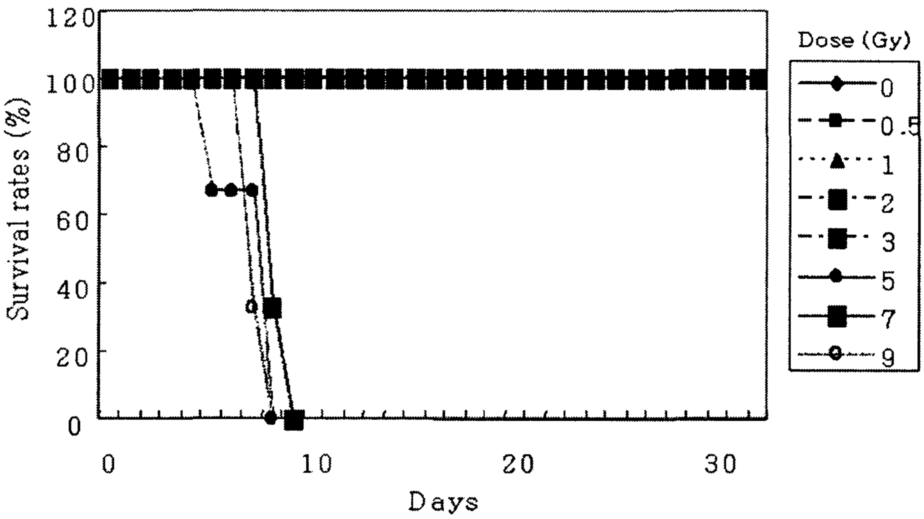

Fig. 1. Survival rates of *Apodemus agrarius* after gamma irradiation (n=15/dose).

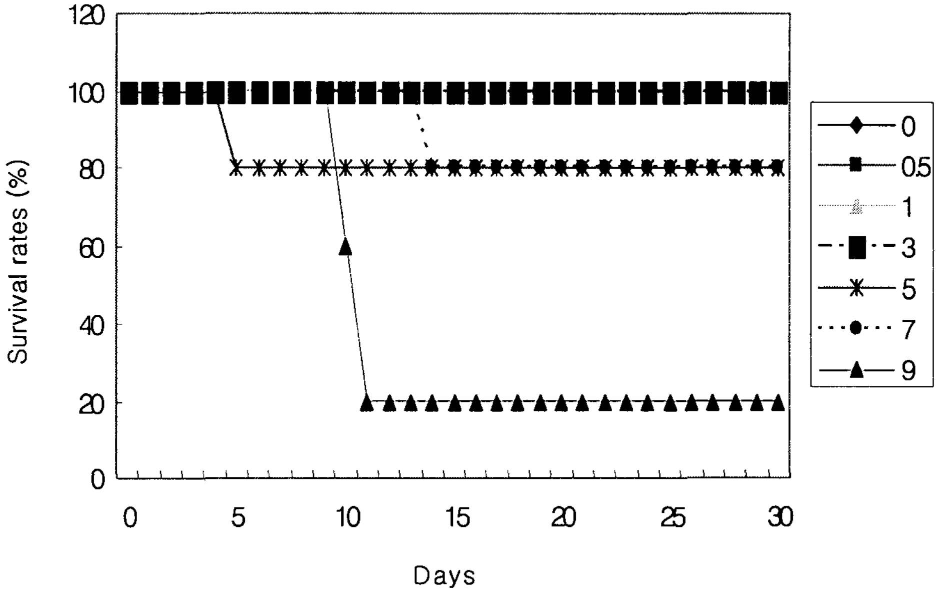

Fig. 2. Survival rates of ICR mice after gamma irradiation.