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Radiation Biology in Space; DNA Damage and Biological Effects of Space Radiation

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Astronauts are constantly exposed to space radiation at a low-dose rate during long-term stays in space. Therefore, it is important to determine correctly the biological effects of space radiation on human health. Space radiations contain various kinds of different energy particles, especially high linear energy transfer (LET) particles. Therefore, we have to study the relative biological effectiveness (RBE) of space radiation under microgravity environment which may change RBE from a stress for cells. Furthermore, the research about space radiation might give us useful information about birth and evolution of life on the earth. We also can realize the importance of preventing the ozone layer from depletion by use of exposure equipment to sunlight at International Space Station (ISS).

key words: space radiation, low dose-rate, high linear energy transfer radiations, relative biological effectiveness

PROGRESS OF SPACE RADIATION RESEARCH

Space science is a new field of study, because human beings will expand more into space in this century. Therefore, the utilization of space and the progresses of space science is certain and more important.

We are now in the progress of constructing an ISS over the next several years. Many kinds of space experiments are scheduled in the ISS. In particular, studies about space radiation might reflect on the safety of life on earth and basic science about the birth and evolution of life. Exposure during space flight consists of low-level back- ground components from space radiation, occasional intense-energetic solar-particle events, periodic passes through geomagnetic-trapped radiation, and exposure from possible onboard nuclear-propulsion engines. For longterm stay in space, we must protect human health from space radiation. To assess the total permissive doses, we aim to obtain the exact RBE value of space radiation [1]. Chronic exposure to space radiation, including heavy ions and other high-LET particles, will be the ultimate limiting factor for the maximum permissible doses [2].

Space radiations of high-LET may induce serious DNA damage compared with radiation exposure on the ground. In recent years, the dose rate of space radiation was estimated at about 1 mSv per day by physical monitoring [3]. This value is almost 1,000 times higher than that on the surface of the earth, because the earth is protected from a high level of space radiation by the ozone layer, atmosphere chemicals and the magnetic field of the earth. To confirm the RBE of space radiation, many space experiments have been per formed [1].

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Chronic exposure at a low dose-rate is a popular expectation in space. On the ground, such conditions are also popular in high background areas, working areas of nuclear power plants and in the medical field. Therefore, studies about radiation environments are very important even on the ground. Of course, most radiation biologists well understand that the RBE of space radiation is different from those from any radiation sources on the ground. Quite small amounts of radiation exposure can bring greater benefits for health than un-irradiated cases. It is referred to as hormesis. Further studies are necessary, as we have no suitable explanation for such differences. Recently, we found that pre-chronic irradiation at a low dose or a low dose-rate induces a radioadaptive response in cultured human cells and mice. Pre-irradiation diminished the biological effect induced by challenging radiation [4,5]. A radioadaptive response depends on an appropriate dose called a window at a specific interval of time. Some reports described that there is radioadaptation in X, γ, and β rays, but not in thermal neutron and high-LET radiation. As the mechanism of radioadaptation, this phenomenon requires enzymatic reaction poly ADP-ribosylation, RNA synthesis, protein synthesis, p53 function and protein-phosphorylation by protein kinases. Our group reported that DNA-PK activity might play an important role in the depression of apoptosis induced by chronic pre-irradiation with a low dose-rate of γ-rays in cultured cells and mouse spleen [5].

For the detection of the harmful influence of cell killing and mutation, the radiation sensitive mutants are very useful. Recently, many kinds of radiation sensitive mutants have been isolated, from microorganisms to human cultured cells. The mechanisms of cell death and mutation have been established at the molecular level. In addition, these biological effects have been found to be induced by indirect processes among cells, known as the bystander effect. Furthermore, a microbeam

apparatus can be used to establish a clear mechanism for the bystander effect. This approach is also very important in understanding the RBE of space radiation.

Studies on cellular stress response have recognized several pathways of signal transduction; a classic pathway is the gene induction of heat shock proteins [6,7]. In space experiments, HSP72 was induced in the muscle, skin and spleen of all flown goldfish as compared with the control [8]. From signal transduction after stress, another pathway focuses on phosphorylation cascades represented by mitogen-activated protein kinase (MAPK) pathways [9], and the third involves the *p*53-centered signaling events in the nucleus. It is well known that p53 contributes to the induction of apoptosis, cell cycle arrest and DNA repair after genotoxic or non-genotoxic stresses [7]. The p53 functions have important roles in the gene stability of the cells [10] during cancer initiation and progression. In contrast, mutation type *p*53 cells have been found to acquire the nature of high mutability [11]. From these points of view, the *p*53 gene is a so-called tumor suppressor gene. DNA damage induces a series of chemical chain reaction including *waf*1, *gadd*45 and *bax* gene expression. We assume that the *p*53-centered signal transduction pathways induced by space radiation might contribute to the gene stability of cells to protect against carcinogenesis. At the present stage, we cannot neglect the possibility that *p*53-centered signal transduction is induced by not only space radiation but also microgravity. Results obtained from space experiments will provide very important knowledge for the adaptive mechanisms of astronauts in space. Therefore, more basic science in cellular signal transduction becomes important in space radiation biology.

When we aim for the success of space experiments, we must establish complete procedures of whole experiments on the ground, because we must put them into the hands of the crew in space. Therefore, we must develop procedures as simple as possible for space experiments. At the same time, we should prepare the development of special equipment, because we cannot take usual equipment into a laboratory in the ISS. Simple handling for organisms and experimental techniques is necessary for the crew. For our previous space experiments, we made special bags to mix 3 kinds of solution for chemical reaction or the growth of microorganisms, because it is difficult to measure the exact volume of solution and mix them in microgravity environment [12,13]. In addition, we made a new syringe without a needle for slime mold in space [14]. Thus, we should devise new equipment when we aim for success in each space experiment.

On the other hand, the common facilities for many principle investigators should be prepared by NASA, ESA and NASDA. To clarify the effects of microgravity, a centrifuge facility (CF) in the ISS is desired. At present, the scheduled CF is a large scale piece of equipment which creates a gravity of 0.01-2.00 g. This CF contains a Glove Box, Advanced Animal Habitat, Plant Research Unit, Aquatic Habitat and Cell Culture Unit. Of course, we must prepare exactly the same experimental facilities in a microgravity laboratory. However, the construction in the ISS will depend on economical conditions.

SAFETY LIFE IN SPACE

One of the characteristics of space radiation is a radiation environment at a low dose and a low dose-rate. From past space experiments by physical dosimetry, the average of dose rate was about 1 mSv per day [3]. In contrast, we are always exposed to about 0.006 mSv per day of natural radiation on the ground. The recommendation of ICRP is 1 or 50 mSv per year for the public and radiation workers, respectively. At this stage, we have schedules for space stays of about 90 days in the ISS. We must establish the maximum period of stay in the ISS from the aspect of health for space crews against space radiation. Space radiations contain a mixture of low-LET radiation and high-LET radiation such as Fe, Ne and neutron. High-LET radiations show wide tracks and induce serious DNA damage over a wide range in comparison with the tracks of the low-LET radiations [15]. In general, the components of high-LET radiations are dependent on solar activity. The biological effect of low-LET radiations is low when given to organisms at a low dose-rate. However, neutron at a low dose-rate brings a rather serious effect. Such a phenomenon is known as a reverse dose rate effect [16,17]. In the case of a low dose and a low dose-rate, we cannot neglect the bystander effect from neighboring cells irradiated with microbeams.

If microgravity enhances the RBE, we must reconsider the RBE of space radiations. Further experiments are required for a correct answer. Mostly high-LET radiations such α a-ray, neutron and heavy particles show a high RBE. Space radiation increased the deletion mutation type instead of a base substitution in the *rpsL* gene in yeast *S. cerevisiae* spores after a 40 day flight on Mir station [18]. A long stay in space inducd chromosome aberrations in the lymphocytes [19,20]. The types of chromosomal aberrations, such as dicentrics and rings, can indicate the type of damage produced with radiation quality which induces baseand deletion-types of mutation. In future, we will examine the gene expression and the adaptation of human cultured cells in a space environment.

In recent years, some contradictory data about the effects of microgravity on radiation-induced biological responses in space experiments have been reported. There are three kinds of reports. One is microgravity enhanced radiation sensitivity. The abnormal development of the insect *C. morosus* from egg to adult insect [21] and the mutation frequency of the radiation-sensitive *D. melanogaster* [22] were reported. Another is the decrease of radiation sensitivity by the use of *D. radiodurans* [23]. The third is no effect of microgravity on radiation-induced incidences in *E. coli* [24-26], *D. discoideum* [14], *S. cerevisiae* [26,27] and human cells [24]. In addition, no effect of microgravity was reported by us involving *in vitro* experiments at the stage of ligation of DNA strand breaks [12] and DNA synthesis

with methylating damaged template DNA [13]. These findings suggest that space radiation may depress the recovery of DNA damage induced by space radiation. From these results, we need to study the RBE of space radiation more. Finally, we want to provide data to maintain the health of the crew based on the analysis of mutation and chromosomal aberration after exposure to space radiation over a long term. To keep the crew healthy, the maximum permissible period of stay in space should be determined from the investigation of the RBE of space radiation. In addition, we expect that the data presented here, from various aspects of radiation research, will provide physiological protection from the serious influences of space radiation during a long stay in space.

FINAL GOAL

Keeping the safety of life on earth

Study in the effects of solar ultraviolet light (UVB) on plants is very important from the view of ozone depletion, a serious global problem, as plants are continuously exposed to solar UV during the daytime. For example, UVB induced the blackening of bananas. However, when we irradiated the banana with white light immediately after UVB irradiation, the blackening was inhibited. These results mean that the blackening might be induced by the formation of pyrimidine dimers produced by UVB. Therefore, the inhibition of blackening may be the result of photoreactivation. When we irradiated with UVB at a high dose of more than 10^5 J/m^2, the photoreactivation was depressed. It is supposed that bananas do not have the full repair capacity for too much UVB-induced photo-damage. These experimental results suggest that there is a limitation of photo-repair activity in bananas. If the ozone layer disappears as a result of advanced culture by human beings in the future, we are afraid that most plants will disappear from the earth. Therefore, we must obtain information about the kinds of plants that can live even without the ozone layer. Space experiments in the ISS might produce a new science to protect the earth.

Further knowledge about the birth and evolution of life on earth

The synthesis of organic chemicals is commonly believed to have happened as the origin of life on the earth about 4 billion years ago by solar ultraviolet light. At that time, the atmosphere is believed to have been anaerobic and consist of no ozone layer. Therefore, the energy from solar UV was stronger than the present. It is likely that such strong UV would produce organic chemicals which are the first stage of the origin of life. Such a hypothesis will be examined as a space experiment at the exposure facility on the ISS. We expect the success of these experiments.

There is another interesting story concerning the relationship between sunlight and life. At the early stage of life, plant organisms carrying photosynthesis activity were believed to be born, at which stage, the atmosphere on the earth might have been changed to an aerobic one. Then, the ozone layer might have been produced by UV and cut out UV of short wave lengths. After which, life, firstly plant and then animal, moved to land from water. This hypothesis will also be demonstrated by space experiments. It is from these aspects that we aim to construct the exposure facility on the ISS.

Most life on the earth has DNA repair mechanisms. Excision repair mechanisms act against UV damage. In addition, repair mechanisms for ionizing radiation is understood to act against DNA damage produced by oxygen radicals and for getting oxygenic respiration necessary for life. Thus, possession of DNA repair mechanisms is a very important subject for basic science. The research about space science may give important information for basic biology.

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