Studies about Space Radiation Promote New Fields in Radiation Biology

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Space radiation / Low dose-rate / High linear energy transfer radiations / Relative biological effectiveness

Astronauts are constantly exposed to space radiation of various types of energy with 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. Studies about biological effects at a low dose and a low dose-rate include various aspects of microbeams, bystander effects, radioadaptive responses and hormesis which are important fields in radiation biology. In addition, space radiations contain high linear energy transfer (LET) particles. In particular, neutrons may cause reverse effectiveness at a low dose-rate in comparison to ionizing radiation. We are also interested in p53-centered signal transduction pathways involved in the cell cycle, DNA repair and apoptosis induced by space radiations. We must also study whether the relative biological effectiveness (RBE) of space radiation is affected by microgravity which is another typical component in space. To confirm this, we must prepare centrifuge systems in an International Space Station (ISS). In addition, we must prepare many types of equipment for space experiments in an ISS, because we cannot use conventional equipment from our laboratories. Furthermore, the research for space radiation might give us valuable information about the birth and evolution of life on the Earth. We can also realize the importance of preventing the ozone layer from depletion by the use of exposure equipment to sunlight in an ISS. For these reasons, we desire to educate space researchers of the next generation based on the consideration of the preservation of the Earth from research about space radiation.

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 the Earth and basic science about the birth and evolution of life1). Exposure during space flight consists of low-level background 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 long-term stay in space, we must protect human health from space radiation. To assess the total permissible doses, we aim to obtain the exact RBE value of space radiation2). Chronic exposure to space radiation, including heavy ions and other high-LET particles, will be the ultimate limiting factor for the maximum permissible doses3). 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 monitoring4). 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 performed5).

ADVANCE OF SCIENTIFIC BACKGROUND IN RADIATION RESEARCH

Recent progress in life science is remarkable from the
aspect of the molecular level especially in the use of DNA research techniques. In radiation biology, too, new knowledge has been established from the molecular mechanisms to replace old understandings. These new findings can be accepted in space radiation biology (Fig. 1). Many kinds of repair genes for UV and radiation-induced damage, from bacteria to human, have been cloned in the past twenty years. Then, the roles of their gene products have been analyzed at the molecular level. These findings contribute to the understanding of radiation sensitivity, DNA repair, cell death, mutation, abnormality, aging, carcinogenesis and genetic diseases.

Most studies about radiation effects have been performed with acute exposure to radiation. In the space environment, however, such acute radiation is unable to be considered. In contrast, chronic exposure at a low dose-rate is a popular expectation in space. On the ground, such conditions are 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\(^5,6\). 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, \(\gamma\), and \(\beta\) 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. Furthermore, this is a cross-reaction between DNA damaging agents and other cytotoxic stresses such as heat. As another mechanism, the radioadaptation may be brought on by DNA repair, antioxidants or protective proteins induced by pre-irradiation. 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 \(\gamma\)-rays in cultured cells and mouse spleen\(^6\).

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.

Almost all organisms exhibit essential recognition systems for environmental changes. Studies on cellular stress

<table>
<thead>
<tr>
<th>Space radiation</th>
<th>Low dose</th>
<th>Adaptive response</th>
<th>Low dose-rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dosimetry</td>
<td></td>
<td>Microbeam</td>
<td></td>
</tr>
<tr>
<td>Shielding</td>
<td></td>
<td>DNA damage</td>
<td></td>
</tr>
<tr>
<td>Bystander effect</td>
<td></td>
<td>Cell cycle</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Signal transduction</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Genetic instability</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Apoptosis</td>
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<td></td>
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<td>DNA repair</td>
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<td>Cancer</td>
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<td></td>
<td>Mutation</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Chromosomal aberration</td>
<td>Microgravity</td>
</tr>
</tbody>
</table>

Fig. 1. The possible contribution of radiation research on the ground to the biological effects of space radiation.
response have recognized several pathways of signal transduction; a classic pathway is the gene induction of heat-shock proteins\(^7\,\!^8\)\. In space experiments, HSP72 was induced in the muscle, skin and spleen of all flown goldfish as compared with the controls\(^9\)\. On the other hand, it has been reported that the space environment did not induce the transcripts of HSP70 or HSP90 in rat testis\(^10\)\. Therefore, further experiments are necessary to determine whether the space environment really causes HSP accumulation. From signal transduction after stress, another pathway focuses on phosphorylation cascades represented by mitogen-activated protein kinase (MAPK) pathways\(^11\)\, and the third involves the p53-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\(^12\,\!^14\)\. The p53 functions have important roles in the gene stability of the cells\(^15\) during cancer initiation and progression. In contrast, mutation type p53\(^\text{mp}p53\) cells have been found to acquire the nature of high mutability\(^16\)\. From these points of view, the p53 gene is a so-called tumor suppressor gene\. DNA damage induces a series of chemical chain reaction including waf1, gadd45 and bax gene expression. We assume that the p53-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 p53-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.

**SEEKING THE SAFETY OF 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\(^9\)\. 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\(^17\)\. 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\(^18\,\!^19\)\. 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 as \(\alpha\)-ray, neutron and heavy particles show a high RBE\. We studied fixed human cultured cells to estimate DNA damage induced by space radiations in a Space Shuttle (9 days) and a Russian space station Mir (40 days)\. 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\(^20\)\. A long stay in space induced chromosome aberrations in the lymphocytes\(^21\,\!^22\)\. The types of chromosomal aberrations, such as dicentrics and rings, can indicate the type of damage produced with radiation quality which induces base- and 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\(^23\)\ and the mutation frequency of the radiation-sensitive (mei-41) D. melanogaster\(^24\) were reported. Another is the decrease of radiation sensitivity by the use of D. radiodurans\(^25\)\. The third is no effect of microgravity on radiation-induced incidences in E. coli\(^26\,\!^28\)\, D. discoideum\(^29\,\!^30\)\, S. cerevisiae\(^28\,\!^31\) and human cells\(^32\)\. In addition, no effect of microgravity was reported by us involving in vitro experiments at the stage of ligation of DNA strand breaks\(^32\) and DNA synthesis with methylating-damaged template DNA\(^33\)\. 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 (Fig. 1), will provide physiological protection from the serious influences of space radiation during a long stay in space.

DEVELOPMENT OF HARDWARE IN THE ISS

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\(^2,3\). In addition, we made a new syringe without a needle for slime mold in space\(^2,3\). 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.

OUR GOAL

(1) Keeping the safety of life on the Earth

Study in the effects of solar ultraviolet light (UV-B) 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, UV-B induced the blackening of bananas. However, when we irradiated the banana with white light immediately after UV-B irradiation, the blackening was inhibited. These results mean that the blackening might be induced by the formation of pyrimidine dimers produced by UV-B. Therefore, the inhibition of blackening may be the result of photoreactivation. When we irradiated with UVB at a high dose of more than \(10^5 \text{ J/m}^2\), the photoreactivation was depressed. It is supposed that bananas do not have the full repair capacity for too much UV-B-induced photodamage. These experimental results suggest that there is a limitation of photorepair 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.

(2) Further knowledge about the birth and evolution of life on the 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.

(3) Success of space experiments

When we aim for the success of space experiments in radiation biology, we must prepare the whole procedure of the experiments. The most important preparations are to
establish the complete experiments on the ground. We must keep in mind the making of simple handling for organisms and experimental techniques. We must develop new specific equipment and simple protocol, as all procedures are performed by space crews.

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REFERENCES


