Technologies and Analyses Using Medaka to Evaluate Effects of Space on Health

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Abstract

Operation of the International Space Station (ISS) has begun. It is important for astronauts to stay healthy and comfortable in space. Reducing influences of environmental stress on astronauts during space flight is a subject of space medicine. With this background, we at the Japan Aerospace Exploration Agency (JAXA) Space Biomedical Research Office (J-SBRO) conduct research to understand and address the effects of the space environment on human health.

Although the abovementioned research is of great importance to human health, it is often very difficult to verify by clinical research alone. Especially the effect of radiation is one issue becoming increasingly important due to the continuous accumulation of space radiation during long-duration stays in space. Astronauts lose bone density and calcium metabolism during space flight, and these phenomena cause serious problems for astronauts in standing or walking when they return to the 1G environment of Earth. As the time spent in space gets longer, continuous accumulation of exposure to space radiation becomes increasingly important during the space flight in both generational and transgenerational effects. In addition, there are several reports on the changes to the immune function in space or on sleep disturbance of both crew members and ground personnel (Larios-Sanz et al., 2007). Thus countermeasures for these problems are required promptly. The purpose of the space medicine is to reduce the influences of the space environment and improve performance associated with an astronaut’s long stay in space. To achieve this purpose, we dedicate ourselves to understanding and reducing the influences of the space environment on the human health by establishing a method to solve and clarify the mechanisms of those medical problems. In such background, we, Japan Aerospace Exploration Agency (JAXA) Space Biomedical Research Office (J-SBRO), are trying to understand and address the effects of the space environment on human health.

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Introduction

The full-scale ISS operation has begun. Japanese astronauts have started long-duration missions aboard the ISS. With these astronauts staying in space, it is important to keep their activity in space healthy, safe and comfortable. Understanding the influences of environmental stress on astronauts during space flight is a major focus of space medicine. Below, here we show a graph of the adaptation to the space environment which was drawn in 1994 by the US space experiment Skylab mission in the 1970s (Fig. 1). During space flight, astronauts face a clinical horizon in most of the physiological phenomena at a certain stage and later adapt themselves from the Earth-normal condition (1G environment) to the space-normal condition (0G environment) afterwards. For example, neurovestibular problems occur in the first few days and the cardiovascular system changes within 1.5 months. However, some physiological alterations accumulate continuously during stays in the 0G environment. Astronauts lose bone density and calcium metabolism during space flight, and these phenomena cause serious problems for astronauts in standing or walking when they return to the 1G environment of Earth. As the time spent in space gets longer, continuous accumulation of exposure to space radiation becomes increasingly important during the space flight in both generational and transgenerational effects. In addition, there are several reports on the changes to the immune function in space or on sleep disturbance of both crew members and ground personnel (Larios-Sanz et al., 2007). Thus countermeasures for these problems are required promptly. The purpose of the space medicine is to reduce the influences of the space environment and improve performance associated with an astronaut’s long stay in space. To achieve this purpose, we dedicate ourselves to understanding and reducing the influences of the space environment on the human health by establishing a method to solve and clarify the mechanisms of those medical problems. In such background, we, Japan Aerospace Exploration Agency (JAXA) Space Biomedical Research Office (J-SBRO), are trying to understand and address the effects of the space environment on human health.

Although abovementioned research is greatly important to human health, it is often very difficult to verify by clinical research alone. Clinical studies mainly
come from the estimation of epidemiological and clinical analyses, especially those on the effect of radiation, such as victims of the atomic bomb or people in an area of high radioactivity. As basic research, biodosimetry enables us to quantify the biological effect and treat it, just as countermeasures and studies using model organisms enable us to estimate the long durational and transgenerational effects and reveal mechanisms or fundamental problems. Mutual basic and clinical research is important in generating a synergistic effect in space medicine or in fields related to human health. Thus, we are trying to understand and to reduce the risk of the influences of the space environment efficiently and safely by using model organisms in some part. Humans and model organisms share similar body systems at the cell or molecular level, and individual responses are constructed at the tissue, cell or molecular level, which makes model organisms so effective in the field of research. In addition, model organisms would be used to verify the transgenerational influence in the future. We discuss our planning space experiments using medaka, *Oryzias latipes* after introducing their features.

### Advantages of medaka as a model organism

Small teleosts including medaka are good candidates for model organisms because their features include a short life cycle or productiveness, and their whole genome sequences are mapped so that the genetic analysis is as easy as the mouse (Kasahara et al., 2007; Kawakami, 2007; Higashijima, 2008; Moens et al., 2008). Medaka and zebrafish, with their transparent bodies through the development, have made contributions to clarifying the molecular mechanism in vertebrate morphogenesis such as whole nuclei tracing during gastrulation (Keller et al., 2008; Ma and Raible, 2009). Recently, small fish are being used as a disease model for infection and immune systems, the heart or liver diseases, muscular dystrophies, or even in the individual learning study (Wittbrodt et al., 2002; Furutani-Seiki and Wittbrodt, 2004; Guyon et al., 2007; Okamoto et al., 2008; Martin and Renshaw, 2009; Rocke et al., 2009).

Why are we using medaka as a model for the space medicine, since medaka and zebrafish seem so similar? Medaka, small teleost that lives in fresh water has been established as a model organism (Wittbrodt et al., 2002; Furutani-Seiki and Wittbrodt, 2004). We show the comparison of medaka and zebrafish below (Table). The range of the breeding conditions is wider in medaka than in zebrafish, such as temperature (4-35˚C in medaka and 25-35˚C in zebrafish) or salt level. Also consistent reproductive activity gives fertilized eggs with fully active testis. This means it is easier for medaka than for zebrafish to create a habitat even during unstable transportation. Moreover, medaka have been studied for the effects of radiation since 1940s (Egami and Etoh, 1966; Hyodo-Taguchi and Egami, 1969; Ijiri, 1995). Finally, medaka have strains with transparent skins, which enables observation of internal organs even in the adult (Wakamatsu et al., 2001). This transparency is an important feature when evaluating the phenomena via live imaging. The abovementioned are the reasons that medaka are suitable for experiments in space compared with other small fish.

### Success and tasks from the previous space experiment using medaka

In 1994, four 1-year-old medaka were launched to space for 15 days, and the medaka space experiment was conducted (Ijiri, 1995). This experiment was the first observation of the mating behavior, development and hatching of vertebrates in space. It also revealed that medaka can adapt to both the space and ground environment. It is known that fish represent looping behaviors as swimming patterns during first few days in space, but fry hatched on orbit behaved in normal swimming patterns in space (Baumgarten et al., 1975; Ijiri et al., 1995a). However, it is difficult to verify the influence of the space environment including adaptation of the long-term effects during the 15-day stay in space.

### Table Features of medaka and zebrafish

<table>
<thead>
<tr>
<th>Features</th>
<th>Medaka (<em>Oryzias latipes</em>)</th>
<th>Zebrafish (<em>Danio rerio</em>)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Place of Origin</td>
<td>Japan, East Asia</td>
<td>India</td>
</tr>
<tr>
<td>Category (Class, Order)</td>
<td>Actinopterygii, Beloniformes</td>
<td>Actinopterygii, Cypriniformes</td>
</tr>
<tr>
<td>Size</td>
<td>3 cm</td>
<td>4 cm</td>
</tr>
<tr>
<td>Generation time</td>
<td>12 weeks~</td>
<td>12 weeks~</td>
</tr>
<tr>
<td>Life time</td>
<td>2.5 years</td>
<td>2 years</td>
</tr>
<tr>
<td>Living temperature</td>
<td>4-38˚C</td>
<td>20-35˚C</td>
</tr>
<tr>
<td>Oviposition cycle</td>
<td>Everyday</td>
<td>1-2 /week</td>
</tr>
<tr>
<td>Egg numbers</td>
<td>20-30</td>
<td>200-400</td>
</tr>
<tr>
<td>Days to hatch</td>
<td>7-10 days</td>
<td>2-3 days</td>
</tr>
<tr>
<td>Genome size</td>
<td>800 Mb</td>
<td>1,700 Mb</td>
</tr>
<tr>
<td>No. of Chromosome</td>
<td>48</td>
<td>50</td>
</tr>
<tr>
<td>Polymorphism</td>
<td>1/100 bp</td>
<td>1/1,000 bp</td>
</tr>
<tr>
<td>Temperature sensitive mutant</td>
<td>15% approx. in whole mutation</td>
<td>Low</td>
</tr>
<tr>
<td>Strains</td>
<td>HdrR, HNI, CAB, Qurt, ST3, SK2, HO5</td>
<td>AB, Tü, TL, Wik, Darj, SJD</td>
</tr>
</tbody>
</table>
These medaka experiments were deemed to have not to be involved only in the biological study but to use as a medical application. Hence we consider the medaka space experiment from the point of view of space medicine, and discuss future research based on the aim of space medicine. We would like to bridge basic research and clinical studies through space medicine.

**Technological progresses on live imaging**

Recently, technology concerning imaging has developed remarkably. There are numerous techniques in microscopy, applications, cameras and communication technologies using satellites. Diversification of tools like fluorescent compounds makes huge contributions to measuring enzyme activities, such as lipid metabolism at both the cellular level and in vivo (Farber et al., 2001; Watanabe et al., 2004; Hama et al., 2009). Unfortunately, the space shuttle will retire in 2010 and material transportation to the ISS must rely on rocket vehicles. Therefore, samples returned will be greatly limited and experimental procedures without sample collection will be required in the future. Live imaging may be one of the solutions. Observation will be more general because of not only the limitation of sample return but also the possibility of the non-invasive approach.

The medaka experiment on orbit plans to start in 2011. It is important to resolve the stress response at the molecular, cellular and tissue level analyses, which have been progressing in recent years, and to clarify minute changes in the genome, such as point mutation. Actually, several ground research projects for space medicine have been started to solve these issues. First, we are going to take up a few research projects categorized in two major ways: short-term stress responses and long-term transgenerational effects. Hereafter, we overview research topics using medaka.

**Stress responses during a short period in the space environment**

Living organisms always expose and respond to stresses from the external world -- physical stresses such as temperature, gravity and radiation: stress of the chemicals and the pH: and environmental stresses like the overcrowded situation. Living organisms, including humans, perceive these stresses at a molecular level, respond at the cellular level, and maintain their lives. Through technologies including live imaging, a part of in vivo phenomena have been come to be appreciated. Fluorescent markers are commonly used in vivo imaging using those materials such as GFP with heat shock promoter. We are concerned with tissue level and individual responses in this review.

**Responses at tissue level**

As for the effects at the tissue level, systematic reaction caused by an autonomic nervous system occurs to protect the body against external stresses. The heart, the intestine and the colon are remarkable tissues controlled by the autonomic nervous system. The gut, especially, is known as an organ that has vagus nerves, and reflects the signals from vagus activation (Kunze and Furness, 1999). Basic and clinical research on effects to intestines from stresses like irradiation have been done (Potten et al., 1994; Buret, 2006). The intestine also has active cell proliferation in addition to the metabolism and absorption of nourishment (van der Flier and Clevers, 2009). Therefore, focusing on the functions of digestive organs by peristaltic movement, cell proliferation, apoptosis, and metabolic alteration can make validating the influence of the space environment, such as radiation, microgravity, and enclosed environment to living organisms, possible.

Even though observation of internal organs has been difficult, they have been analyzed by ultrasonography or contrast medium because of the importance of their functions (Rhodes et al., 1966; Schulze, 2006; Haruma et al., 2008). Thus, we are trying to evaluate the heartbeat, peristaltic movement, or enzymatic activities of metabolism using transparent medaka with live imaging (Fig. 2). From these images, the heart beat and peristaltic movements are both distinguishable in fry and adult. It is important to reduce stresses of fish, we use agarose mold to fix medaka and take movies of ventral side for 5 minutes maximum. We are exporting imaging data of heart beat rate or peristaltic movement as a numerical value. The effect of space radiation and the space environment on the internal organs will be evaluated by this method as well.

**Fig. 2.** Live imaging of the heartbeat and peristaltic movement using medaka

(A) Medaka strain SK2 is possible to visualize the internal organs (g: the gill, h: the heart, l: the liver, gu: the gut and f: the anal fins, respectively) even in the adult under the microscope. (B and C) Images of the heartbeat. White arrowheads show the chamber wall between atrium in 2 different time points. (D-G) Images of the peristaltic activity in adults (D and E) and fry (F and G). Asterisks show the position of the contraction between 2 time points.
**Behaviors of individuals by space environment**

As for gravity, there is a report that the medaka sank and did not swim more than 24 hours after the 15-day space flight (Ijiri, 1995). There is another report on the medaka experiment that the eggs rotation in the chorion were less happened under the microgravity environment from the STS-107 experiment in 2003 (Niihori et al., 2004). It was also shown that invitation processes of the motility and the control of its posture were also decreased in medak fry under the micro gravity environment. These reports suggested the behavior of eggs, fry and adult would change under the microgravity environment.

JAXA is developing the aquatic animal habitation module (Aquatic Habitat: AQH), which will enable breeding small fish for about 3 months in the ISS. Time to reproduction is generally 3 months in medaka so it is possible to see the phenomena in the F2 generation of medaka launched to the ISS. Moreover, it is important to verify the biological influences, such as their behavior in the space environment long term.

**Researches related to the transgenerational effect**

The space environment that is just outside the geomagnetic field always pours down space radiation with proton and heavy ion rays. Even though the ISS has some protection from the radiation, it is said that astronauts are exposed to 0.5-1.0 mSv per day in the ISS. This is almost the same as half year of irradiation on the ground (Yasuda, 2009). One of the most serious problems as an influence of long-term stays on orbit is the rising rate of cancer. However, it is very difficult to detect the phenotype as a cancer especially in low dosage irradiation. It is also difficult to use an astronaut as a test subject, because of the variation of the genomic background of humans in addition to the small sample numbers. As well, the main reason for the cancer is the accumulation of damage to the genome such as mutations by irradiation, for example. Therefore, we are trying to detect the effects of radiation based on the mutation on the genome, which is more sensitive and quantitative than the cancer rate.

**Transgenerational effects at genomic level**

There are two types of genomic influences: the macro level as an abnormal chromosome and the micro level like a single base mutation. Chromosomal abnormalities are found from the alterations of the fertilization rate or hatching rate, and actually, there was a report that showed no difference in the survivability of fertilized eggs over 15-days of space flight (Ijiri, 1995). However, micro alterations in the genome have not been indicated yet, it is necessary to verify the genome influences of the microsatellite by an inbred medaka strain which is revealed the genomic sequence. It is known that higher spontaneous mutation frequencies occur in microsatellite than in coding genes and such repeat sequences are hyper-mutable by irradiation both in somatic and germ-line cells (Thibodeau et al., 1993; Mairs et al., 2007).

The research to examine the possible effect of low-dose radiation on microsatellite loci has considerably increased both in the number of studies and in the number of organisms including medaka (Tysusko et al., 2007). In this experiment, male medaka from space are crossed with ground female to evaluate the effects on testsis as a transgenerational effects or evaluated the phenotype in genome in the testsis by staining.

**Responses at a cellular level**

Usually cellular responses such as DNA repair and apoptosis occur to prevent carcinogenesis when living animals are exposed to radiation. Both wild type and medaka mutants that affected restoration of γ-ray irradiation effects has been analyzed the effect of radiation during embryogenesis (Aizawa et al., 2004; Aizawa et al., 2007). The cell lines derived from those medaka mutants shows abnormal stress response in DNA repair, check point and apoptosis and the molecular bases of the DNA damage signal transduction network is investigated (Hidaka et al., 2009). Recently, the irradiation experiment has been conducted by various kinds of beam rather than X-ray or γ-ray irradiation as before (Kuhne et al., 2009). It is necessary to examine the effect of long-duration low doses of ionizing radiation for modifying space radiation in the future.

p53 is a tumor suppressor protein and known as ‘Guardian of the Genome’. It plays an important role in DNA repair, cell cycle control and apoptosis. The loss of function type mutations in the medaka p53 gene of were identified by reverse genetics approach (Taniguchi et al., 2006). They will be very sensitive strains to examine the frequency of the mutation in medaka genome in testsis during space flight espacially taken advantage of the genome size of medaka which is as half as zebrafish. Although the observation from the wild type cells may underestimate the actual damage to the cell, using p53 mutants enables the detection of cell damage in the genome without DNA repair or induction of apoptosis. Moreover, the testsis is the most efficient organ to evaluate the irradiation effect even compared with the ovary. These facts suggested that it might be possible to detect the irradiation phenotype at a germ cell level with this study. This project is planned in the ISS.

**Closing remarks**

The effects of space flight on health and analytical approaches using medaka are shown in this review (Fig. 3). Physiological phenotypes occur mainly during space flight because of the adaptation processes to space and the ground environment. Radiation effects may remain or even increase after landing because of the accumulation of mutation or degeneration in the body. Therefore, medaka is a model organisms in analyzing the transgenerational effect of radiation because of its shorter...
life cycle and the genetically-modified approaches.

We have been discussing the potential of medaka so far, it is necessary to begin with systematic clarifying the availability of medaka as the model organism in space medicine as a next step. Therefore, to unify and apply the medaka research in Japan, the “medaka consortium (secretariat: Prof. Hiroshi Mitani)” has stood up among several laboratories in universities or research institutes including JAXA in 2009.

The study of evaluating the effects of radiation on human health is going to be important in the future because the targets of exploration will transition from the ISS to the moon and Mars, and the target environment is going to follow. We have described this issue from the introduction to the current picture of the space medicine experiment using medaka. The aim of basic research using model organisms or cell culture is to understand the environment of space for the healthcare of astronauts. However, as researchers involved in space medicine, what we cannot forget is the research direction. Thinking about the astronaut is the same as thinking about the people living on Earth, which is also a space environment. Beyond the astronaut, this research is to promote a healthy life for the humankind, such as the improvement of the living environment or drug screening for the protection of radiation.

Acknowledgement

We extend our thanks to Dr. Toshiko Ohta for her constructive advice and criticism of this paper.

Fig. 3. Health effects in space, analytical targets and methods using medaka
Several physiological changes and radiation impacts are picked up in this review as phenomena to be revealed using medaka with analytical techniques. Each phenotype is connected to corresponding target and method to be used.

<table>
<thead>
<tr>
<th>Health effects in space</th>
<th>Analytical targets</th>
<th>Methods</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stress responses in the space environment</td>
<td>Peristalsis</td>
<td>Peristaltic movement analysis</td>
</tr>
<tr>
<td>Reduction in autonomic nervous system regulation</td>
<td>Heart beat</td>
<td>Heart rate spectral analysis</td>
</tr>
<tr>
<td>Muscle atrophy</td>
<td>Pathology</td>
<td>Microarray, Proteome analysis,</td>
</tr>
<tr>
<td>Alteration in digestive function</td>
<td>Chemical assay</td>
<td>ATPase activity assay</td>
</tr>
<tr>
<td>Immunological deterioration</td>
<td>Cell dynamics</td>
<td></td>
</tr>
</tbody>
</table>

| Responses related to transgenerational effects       | Genomic mutation            | Genome sequence,                |
| Higher risk of cancer (mutation risk)                | Apoptosis                    | High-resolution melting analysis|
| Transgenerational effect (Gonadal effect)            | DNA repair                   | TUNEL, Micro-nuclei             |

|                                      | Testis abnormality           | Sperm formation analysis,       |
|                                      |                             | GFP/DS-Red -sperm              |

References


