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Future role of vitamin C in radiation mitigation and its possible applications in manned deep space missions: survival study and the measurement of cell viability

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ABSTRACT

Background: Astronauts will be exposed to both chronic space radiation and acute high doses of energetic radiation of solar particle events in long-term deep space missions. The application of radioprotectors in space missions has basic limitations such as their very short time window as well as their acute toxicity and considerable side effects. The aim of the present study was to investigate the potential radiation mitigation effect of vitamin C that is known as an effective antioxidant and free radical scavenger. ***Materials and Methods:*** One hundred twenty male Wistar albino rats weighing 250-300 g were randomized into the following study groups: I, control; II, Only exposure to gamma-radiation ($LD_{50/30}$); treated with a single dose of vitamin C, III, 1h before irradiation, IV, V and VI, 1h, 12h and 24 h after irradiation. Measurement of cell viability and proliferation was also performed by using MTT cell proliferation assay. ***Results:*** The survival rate in animals received vitamin C 1h, 12h and 24h after irradiation were 55%, 60%, and 80%, respectively. The viability of cells in animals received vitamin C 1h, 12h and 24h after irradiation were 94.9%, 99.0%, and 100%, respectively. The viability of the cells in animals only exposed to gamma rays was 50.1%. ***Conclusion:*** These findings reveal that a single dose of vitamin C can potentially be used up to 24 hours after exposure to reduce the detrimental effects of high levels of ionizing radiation in cases such as the occurrence of currently unpredictable solar particle events.

Keywords: *Space radiation, radiation mitigation, vitamin C, astronauts, survival, cell Viability .*

INTRODUCTION

Earth's geomagnetic field protect us from Galactic Cosmic Radiation (GCR) and energetic solar flare protons. However, astronauts who participate in long duration exploratory missions will not benefit from this natural radiation shield. Astronauts exposed to GCR in spacecrafts with inadequate shielding during solar minimum can exceed current guidelines set for astronauts' exposure. Energetic Solar Particle Events (SPEs) which are unpredictable in nature, are potentially life threatening especially for astronauts inadequately protected. Solar particle events (SPEs) are primarily composed of low to moderate energy protons while isotropic galactic cosmic radiation is dominated by protons with a wider energy range. Although more efficient radiation protection on Earth can be easily achieved through increased shielding, efficient shielding against space radiation may not be easily achievable due to factors such as extremely penetrating nature of space radiation and limitations in shields' weight and cost. It should be noted that the cost of a kilogram mass transported from Earth to the Moon is estimated to reach 80,000 USD. Considering these limitations we have previously discussed that due to limitations of physical shielding and advantages of biological shielding, only a smart combination of physical and biological shielding may solve the problem of possible exposures to intense radiation in long-term space missions [1, 2].

Although numerous compounds have been studied as potential radioprotectors, their in vivo use is limited by negative factors such as acute toxicity and side effects. Vitamin C is a known antioxidant and free radical scavenger [3].In animal models, it has been reported that vitamin C can prevent the adverse effects of whole body radiation through increasing the antioxidant defense systems in the liver and kidney of irradiated animals [4]. Different aspects of the radioprotective effects of vitamin C have been previously investigated by Mozdarani *et al.* and other researchers [5-9].

It has been reported that some of the antioxidants and dietary supplements can better prevent the detrimental biological effects of radiation when applied several days after exposure to radiation [10]. Furthermore, it has been shown that a diet supplemented with antioxidants administered starting 24 h after total body irradiation is more effective than if given soon after the exposure [11]. It was previously reported that for a long term space mission the magnitude of the induced adaptive response of all candidates should be measured by *in vitro* tests and only those who show high levels of adaptive response should be selected as potential crew. The hypothesis was that chronic exposure to higher than on-earth levels of radiation can greatly decrease radiation susceptibility of astronauts and help them better cope with the detrimental effects of the exposure to unpredictable sudden solar flares and coronal mass ejections which cause dramatic increase in radiation flux [12-14].

Appropriate radioprotectors can be used in a wide range of applications including space travel, medical exposures and nuclear accidents [15]. We have previously reported that some basic shortcomings such as the very short time window for application of these agents (they must be administered before exposure) and their considerable toxicity limit the administration of traditional radioprotectors as efficient tools for reducing the risk of space radiation [2]. We also previously hypothesized that finding appropriate radiation mitigators with a post-exposure time window longer than 24 hours will be a cardinal goal in planning future manned space missions [1]. This hypothesis was based on this fact that Solar Particle Events (SPEs) which can expose the astronauts to doses as high as lethal doses ($LD_{50/30}$) are currently unpredictable and they usually continue from several hours to several days. In this light, we believed that by replacing conventional radioprotectors with radiation mitigators with a long post-exposure time window, astronauts will have enough time to evaluate their radiation exposure, before choosing any therapeutic intervention. They can also consult expert scientists on the Earth to make sure if they should use any radiation mitigator. This study was an attempt to investigate if vitamin C can be considered as an efficient radiation mitigator

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which may help astronauts better cope with the detrimental effects of space radiation hours after exposure to relatively high levels of radiation caused by solar particle events [1].

MATERIALS AND METHODS

Animal Setting

One hundred twenty *male Wistar rats* weighted *250–300 g (75–90 days old)* were randomly divided into 6 groups, each one consisting of 20 animals. The animals were kept in special cages with controlled temperature and humidity. Animals were kept under a 12-hour light and 12-hour dark cycle at ambient temperature (21 ± 1 °C) with free access to food and water. The 1st group served as the control. Animals in the 2nd group received only the lethal dose of gamma radiation. The 3rd group received a single dose of vitamin C (400 mg/kg) 1h before irradiation. The 4th, 5th and 6th groups received vitamin C 1h, 12h and 24h after irradiation. Survival of the animals was monitored continuously for 30 days after irradiation. All animal experiments were considered and approved by the Animal Experimentation Ethics Committee of Shiraz University of Medical Sciences prior to commencing work.

Exposure to lethal dose

All animals were exposed to a lethal dose (LD 50/30) of 8 Gy of gamma radiation [16] emitted by a Theratron Phoenix (Theratronics, Canada) Cobalt-60 therapeutic source (dose rate 55 cGy/min) at Radiotherapy Department of Namazi Hospital, Shiraz, Iran.

Survival study

After irradiation rats were moved back to their cages and their survival was carefully monitored three times a day for 30 days.

MTT Assay

All animals sacrificed on day 30. Measurement of cell viability and proliferation was performed on peripheral blood *mononuclear cells* by using MTT cell proliferation assay (ATCC® 30-1010K, American Type Culture Collection, USA). After an overnight incubation, 10 µl of MTT solution was added for an additional four hour incubation, according to the standard colorimetric assay. The absorbance was measured at 570 nm.

Statistical Analysis

Kaplan-Meier survival analysis (SPSS 17.0) was used to evaluate the statistical significance of the differences of survival rates among different groups. A dead animal was counted as 1, whereas alive animals were defined as 0. The differ-ence among the survival rates of the groups was evaluated by the log-rank (Mantel-Cox) test. Log-rank test was considered statistically significant if p value obtained from $\chi 2$ test was less than 0.05. ANOVA followed by post-hoc test was used to assess the statistical significance of the differences of cells' viability in different groups.

RESULTS

Findings of this study showed that vitamin C could serve as an efficient radiation mitigator in this study. Survival curves of the rats treated with vitamin C either 1h before or 1, 12 and 24h after irradiation with a lethal dose (LD) of gamma radiation are shown in figure 1. As shown in this figure, thirty days after irradiation the survival fractions for the control group was100%, while the survival fraction in the animals received $LD_{50/30}$ was only 50%. The survival rate in animals received vitamin C 1h, before irradiation was80%. The survival rate in animals received vitamin C 1h, 12h and 24h after irradiation were 55%, 60%, and 80%, respectively. The log-rank (Mantel-Cox) testshowed that the survival rate in the 2ndgroup that only received the lethal dose was significantly less than that of those treated with vitamin C (p=0.039) either 1h before or 24h (p=0.047) after irradiation. Figure 2 shows the results of MTT assay (the viability of cells in animals received vitamin C 1h before, or 1h, 12h and 24h after irradiation compared to those only exposed to $LD_{50/30}$). As shown in the figure, the viability of cells in animals received vitamin C 1h, 12h and 24h after irradiation were 94.9 %,

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99.0 %, and 100 %, respectively. The viability of the cells in animals only exposed to gamma rays was 50.1%. Tukey test showed that the viability of the cells in the 2nd group that only received the lethal dose was significantly less than that of those treated with vitamin C 1h (p=0.001), 12h (p<0.001) and 24h (p<0.001) after irradiation. Although the survival rate in the 6th group which treated with vitamin C 24h after irradiation was higher than that of those treated with vitamin C 1h or 12h after irradiation, log-rank (Mantel-Cox) could not show any statistically significant difference.

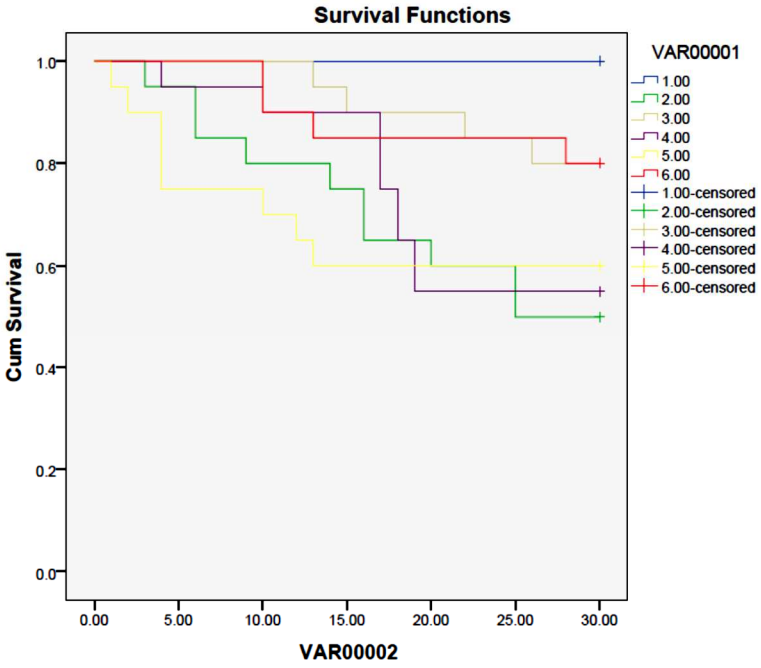

Figure 1. Kaplan Meier survival curves of the rats treated with vitamin C either 1h before or 1, 12 and 24h after irradiation with a lethal dose (LD) of gamma radiation.

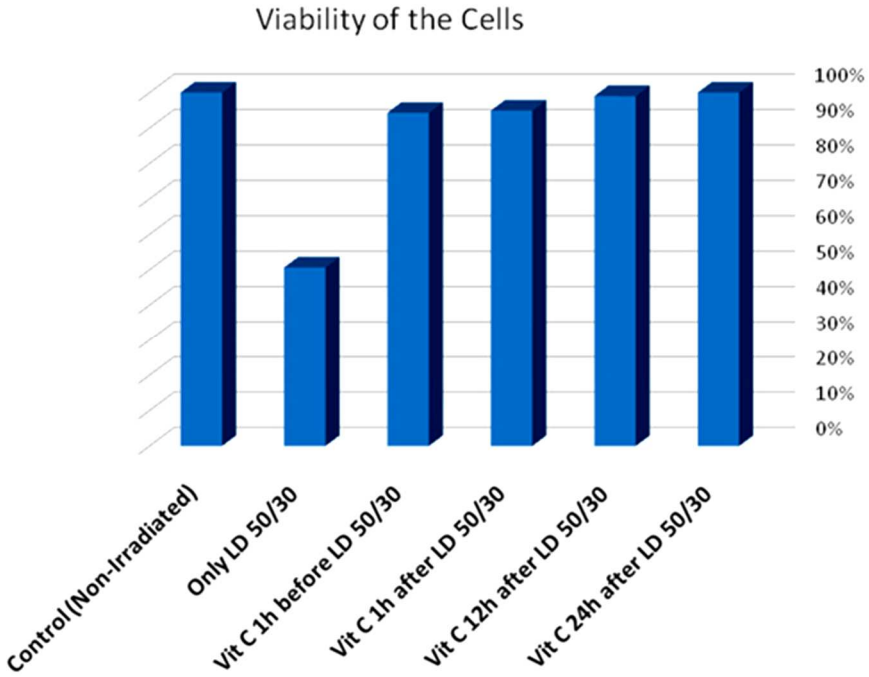

Figure 2. MTT assay shows the viability of cells in animals received vitamin C 1h before, or 1h, 12h and 24h after irradiation compared to those only exposed to $LD_{50/30}$.

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DISCUSSION

The survival rate in animals received vitamin C 1h, 12h and 24h after irradiation were 55%, 60%, and 80%, respectively. These findings clearly lead us to conclude that vitamin C can potentially be used up to 24 hours after exposure to high levels of ionizing radiation in cases such as the occurrence of currently unpredictable solar particle events. In this light, astronauts will have the critical opportunity of evaluating their radiation exposure, before choosing any therapeutic intervention. This long time window even allow them to consult expert scientists on the Earth to make sure if they should use any radiation mitigator. From a general point of view, our findings are in line with the results obtained in other studies on the radioprotective effects of vitamin C such as those indicate that vitamin C can serve as an antioxidant to protect DNA damage caused by exposure to ionizing radiation [17] or the report indicated that pretreatment of mice with vitamin C can significantly reduce the lethal GI damages induced by ionizing radiation [18]. The latter study showed that the survival rate in mice pretreated with vitamin C for three days before exposure to lethal whole body radiation of 14Gy and a subsequent bone marrow transplantation (24 h after irradiation) was 40%. However, post-treatment alone was ineffective in that experiment [18].

Long term manned space missions, may be a few years long, will be planned in a near future. Basic problems such as microgravity and the risk of exposure to high levels of ionizing radiation are still among the major factors that limit the duration of these missions. It has previously been shown that adaptive response, as described above, can significantly decrease the radiation damage in humans after exposure to a low dose [19-23]. Our suggested method was based on our early findings about inter-individual variability in induction of adaptive response [24] which clearly indicated that ground-based adaptive response studies may help us identify subpopulations with the highest magnitude of induced adaptive response. As radiofrequency radiation can also induce adaptive response [16, 25]. We reported that pre-exposure of animals to radiofrequency radiation can lead to increased resistance against subsequent bacterial infections. We suggested that these findings, if confirmed by well-structured human adaptive response experiments, can be used to overcome the problems associated with the risk of infection during any deep space mission.

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Conflict of interest: *Declared none*

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