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Transfer of Radio-Adaptation via Serum: A Preliminary Report

Ehsan Khodamoradi [1], Rasool Azmoonfar [2*], Mohammad Mohammadi [1], Amir Kiani [3], Abbas Haghparast [4], Nasrin Sharafi [1], Loghman Diojan [1]

1. Department of Radiology and Nuclear Medicine, Faculty of Paramedical Sciences, Kermanshah University of Medical Sciences, Kermanshah, Iran
2. Department of Radiology, Faculty of Paramedical Sciences, Hamadan University of Medical Sciences, Hamadan, Iran
3. Associate Professor, Faculty of Pharmacy, Kermanshah University of Medical Sciences, Kermanshah, Iran
4. Associate Professor of Medical Physics, Department of Medical Physics, Faculty of Medicine, Kermanshah University of Medical Sciences, Kermanshah, Iran

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ABSTRACT

Introduction: Adaptive response is one of the important concepts in radiobiology. The present report aimed to transfer the radio-adaptation via serum.

Material and Methods: In total, 50 male adult Wistar rats were randomly divided into 6 groups, including control, serum control, low-dose (100cGy), low-dose/lethal, serum/lethal, and lethal (8Gy). Exposure was carried out by a linear accelerator (Elekta Synergy® Platform) with a 40×40cm field size. The animals were monitored in terms of the endpoints of the survival rate, and at the first stage, the rats were exposed to the low doses of radiation. Subsequently, the serum was injected intraperitoneally under sterile conditions 6 h after low-dose exposure. The Kaplan Meier Survival Curve was used to evaluate the survival rate ($P<0.05$).

Results: There was a significant difference among different groups regarding the survival rates. Moreover, a statistically significant difference was observed between low-dose/lethal and low-dose/serum, low-dose/lethal and lethal, and low-dose/serum and lethal ($P=0.001$). Similarly, there was a statistically significant difference between the control and experimental groups regarding the survival rates ($P=0.001$).

Conclusion: To the best of our knowledge, this method can lead to immunological responses or unknown mechanisms that result in the increased survival adaptive response to subsequent high-dose radiation.

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Introduction

Nowadays, ionizing radiation is widely used in diagnostic imaging and treatment in medicine. Despite the important role of these beams in the diagnosis and treatment of diseases, fear of exposure to low doses of radiation has always been considered, and there is an uncertainty in measuring the biological effects of radiation in this dose range [[1]. Radiation protection during nuclear accidents is essential along with occupational exposures to ionizing radiation with cosmic origin and unwanted exposure [2-4].

Moreover, increased rate of space explorations as long-term projects for new discoveries resulted in astronauts' exposure to cosmic rays, including high-energy protons, heavy ions, and secondary particles which requires protection [5]. Therefore, the protection of astronauts in space is one of the priorities of the National Aeronautics and Space Administration, European Space Agency, and Astronaut Center [6, 7]. Since the use of ionizing radiation in the diagnosis and treatment of diseases is very important, radiation hazards remain an important and undeniable issue [8]. For many years, studies on ionizing radiation have shown that it leads to biological damages and carcinogenesis [9]. In the past 20 years, many studies have been conducted on the radiobiological phenomena, such as bystander effect, abscopal, and the radio-adaptive response which was carried out with ionizing and non-ionizing radiation [10-15]. The adaptive response is a well-documented phenomenon in radiobiology which is important in determining biological responses at low doses of radiation. This term usually means that pre-exposure to low doses of radiation increases radiation resistance after the exposure to higher doses a few hours later [16-18]. Over the past three decades, several investigations reviewed radio-adaptive response phenomena [19-22]. Oliveri et.al, for the first time (1984), showed that when human lymphocytes were exposed to tritium (i.e., thymidine), it would result in the resistance of these cells against cytogenetic damage caused by high dose. According to the results of the aforementioned study, it was called radio-adaptive response [23]. Adaptive radiation response occurs at low doses in all organisms, and this response reduces the harmful effects of DNA damage. It has also a specific window that is defined as the

*Corresponding Author: Tel: +98 813 838 1037; E-mail: Azmoonfar@live.com, r.azmoonfar@umsha.ac.ir

upper and lower dose range, which is usually between 1 and 100mGy [24]. The adaptive response has been investigated with ionizing and non-ionizing radiation [25] in human lymphocytes [26], fibroblasts [27], animal models, and cell culture specimens [23, 28, 29]. Due to the increasing use of ionizing radiation in medical diagnostic imaging and the fear of its effects on the body, many scientists are required to study these effects in low doses (≤ 0.1 Gy); however, there is uncertainty regarding the measurement of its biological effects [1]. Although exposure to ionizing radiation can induce an adaptive response in the dose range from 5 to 100 mGy [30-32], this phenomenon has not been fully identified in low dose radiobiology [33]. Different signaling pathways can be involved in an adaptive response [34-36].

Various mechanisms which are proposed for this phenomenon in different studies include genetic factors [37], up-regulation of hypoxia-inducible factor 1 after low dose exposure [38], and increased protein synthesis [39, 40]. However, the two main important mechanisms related to radio-adaptive response are gap-junction intercellular communication and the release of diffusible signaling molecules [23, 41-43]. T-helper 1 (Th) and T-helper 2 cytokine expression immune responses after low and high dose ionizing radiation can have critical roles in shifting Th1 cells to Th2 [44]. This study aimed to describe the adaptive response among rats in which they received rats' serum at pre-treatment which was exposed to low dose radiation prior to the challenging dose. Subsequently, the rats were compared with those that were just exposed to the challenging dose in terms of the survival rate.

Materials and Methods

Animals

After reviewing the available resources, 56 male adult Wistar rats (weight range: 200-250 g) were included in this experimental-interventional study. The rats were then assigned into cases (n=14) and controls (n=28); moreover, the pre-irradiated rats were randomly divided into 7 groups to separate their serum (Table1).

All rats were kept under controlled humidity, temperature (22±1°C), and lighting (12:12 h light-dark cycle). All animal experiments were approved by the Animal Experimentation Ethics Committee of Kermanshah University of Medical Sciences, Kermanshah, Iran (Ethical code: 3004579). Eventually, the adaptive response of the studied groups, especially those receiving blood serum, compared with that of the other groups.

Irradiation Condition

The exposure is carried out with the Linear Accelerator (Elekta, UK) in the Radiotherapy Department of Imam Reza Hospital, Kermanshah, Iran. Low-dose and lethal irradiation doses were 100cGy and 8Gy megavoltage x-ray, respectively. It should be noted that the irradiation was performed inside the cage (Energy: 6MV, Dose Rate: 460cGy/min, field size: 40×40 cm^2, SSD: 100 cm).

Blood Sampling and Serum Injection Procedure

After 6 h of exposure to a low dose, the serum of the irradiated rats (group 0) was separated and injected into groups 2 and 5. Initially, the rats were anesthetized and the blood was excreted directly from the heart under surgical conditions and sterilization; in addition, the separation of serum was performed under sterile conditions. The extracted blood was placed in Bain Marie for 45 min at 37 °C and then separated by serum centrifugation at 2500rpm and 10min. The serum was injected intraperitoneally under sterile conditions and under the laminar hood. Figure 1 illustrates all the experiment processes.

Survival Study

After 24 h of low-dose irradiation for group 4 and serum injection for group 5, both groups were exposed to the lethal dose. All 6 groups were monitored by experts and death events were followed and registered daily for 30 days. In this study, the survival rate was defined as the percentage of the survived rats after 30 days.

Statistical Analysis

The Kaplan Meier Survival Curve was used to evaluate the survival rate in the studied groups. A P-value less than 0.05 was considered statistically significant.

Table 1. Animal groups

Groups #	Description
0	Rats were exposed to low-dose irradiation, and their serum separated and injected into groups 2 and 5 (n=14). This group is not a case or control.
1	Control group without any serum injection and irradiation (n=7).
2	Control group with serum injection and without irradiation (n=7).
3	Case group was exposed to low-dose irradiation (n=7).
4	Case group was exposed to low dose irradiation, and it was exposed to lethal doses after 24h (n=7).
5	Case group with serum injection, and it was exposed to lethal dose irradiation (n=7).
6	Case group without serum injection and low-dose irradiation. The rats in this group were only exposed to lethal dose irradiation (n=7).

Results

The below line graph illustrates the survival rate of rats in different groups over a period of time (day). It can clearly be seen that group 5 (serum/lethal) had the most survival rate during the study, whereas group 6 obtained the least survival rate of three (Figure 1).

According to Table 1 (supplementary), the first thing to notice is the lack of no death in groups 1, 2 and, 3 during 13, 18, and 22 days since the beginning of the experiment. Moreover, there is a clear similarity among these three groups whose survival rate was 100%. In contrary to these three groups, there is a significant difference among groups 4, 5, and 6. Animal survival data were analyzed using the Kaplan-Meier test (Figure1). According to the results obtained from this test, a significant difference was observed between groups 4 (low-dose/lethal) and 5 (low-dose/serum), groups 4 and 6 (lethal), and groups 5 and 6 (P=0.001). In addition, control and low-dose/serum groups showed a significant difference with groups 4, 5, and 6 (P=0.001).

According to table 2, there is still 100% survival in groups 1, 2, and 3 up to the 25th day. Kaplan Meyer curves revealed that 50% of the population died in groups 3, 4, and 5 on the 13th, 17th, and 22nd days, respectively. The results of this study demonstrated that the exposure of rats to low-dose radiation (100cGy) and then the injection of their serum to another group (group5) which was followed by a later exposure to a high dose (8Gy) 24 h later made the survival rate much better than that in group 4 (Table 2).

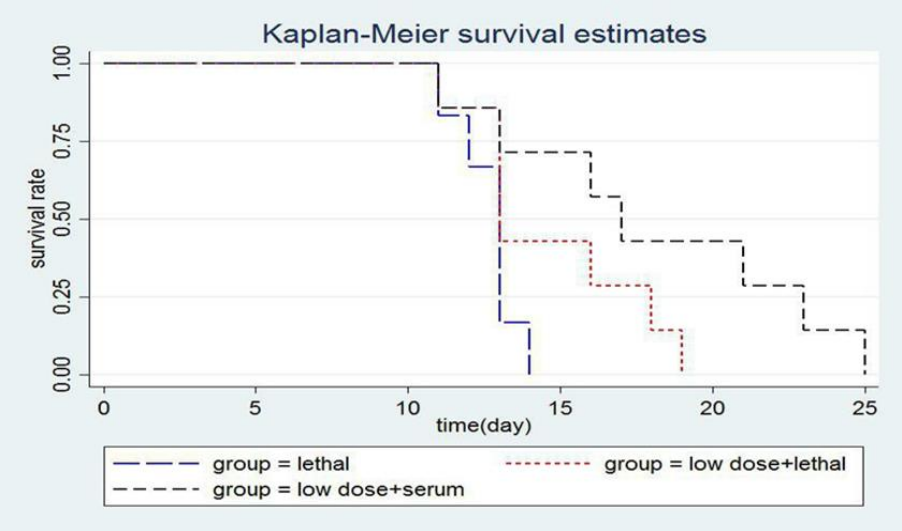

Figure 1. Kaplan-Meier survival curves in low-dose/lethal, serum/lethal, and lethal groups

Table 2. Survival rates in different groups of rats after 13, 18, and 22 days of exposure to the lethal dose (LD 50/30) of X-radiation

Groups #	Name	Challenge Dose(LD50/30)	Survival Rate		
			After 13 Days	After 18 Days	After 22 Days
1	Control	-	100%	100%	100%
2	Serum control	-	100%	100%	100%
3	Low-dose	100cGy	100%	100%	100%
4	Low-dose/lethal	8Gy	42%	14%	0%
5	Serum/lethal	8Gy	71%	43%	28%
6	Lethal	8Gy	16%	0%	0%

Discussion

In the present study, the radio-adaptive response was investigated by injecting the blood serum of irradiated rats with low-energy ionizing radiation into another group. Organisms are affected by various DNA damage agents that occur naturally in the environment; therefore, defense mechanisms have evolved to minimize genetic damage in organisms. Cellular damage as a result of ionizing radiation in low doses is generally extrapolated from observing the effects in high doses which is not very accurate. Radio-adaptation response refers to a group of non-target effects that do not require direct exposure of the target cells and are indirectly affected by radiation. After a challenge dose, the damage is reduced when this mechanism is induced by a previous low dose [20]. The precise mechanism of the

radio-adaptive response is not fully understood [45]. This phenomenon occurs optimally in a small window typically between about 1 and 100mGy for a single low dose rate exposure.

Different evidence suggests that the responses of radiation in low doses are different, compared to high doses [46]. Studies show that the activation of the radio-adaptive response does not occur instantaneously, and it takes about 4-6 h for this effect to be fully activated [47]. Similarly in our study, the interval between exposure time and serum injection was within the same time interval of 6 h. There were no deaths in groups 1, 2, and 3 during the 13th, 18th, and 22nd days and the survival rate was 100%. This result was predictable since the low-dose irradiation does not lead to significant biological damages. Our results indicated a significant change in the survival rate in pre-treated rats by serum, compared to the time when they were exposed to a low dose. Accordingly, it can be concluded that intraperitoneally serum injection induced an adaptive response.

The results of this study are consistent with those in previous studies which illustrated the possibility of the induction of adaptive response after pre-treatment with low-energy ionizing radiation [37, 48, 49] or non-ionizing radiation [50, 51]. Our finding also confirms that pre-treatment of animals with pre-exposure serum significantly increased the radio-resistance of animals. After radiation exposure, the immune system plays an important role in carcinogenesis. It is believed that in the long term, malignant cells can be eliminated by the immunological process. The stimulation of the immune system by low doses of radiation has been reported in mice and rabbits [52]; moreover, in various biological systems, different stress conditions can activate similar defense mechanisms [53]. It is suggested that immune responses could play a critical role in enhancing defense mechanisms and animal survival.

The present study is the first investigation on radio-adaptive response using an intraperitoneally pre-irradiated serum. The present study offers a novel approach to radiation protection, and further studies are suggested to utilize this method to produce quasi-vaccination to protect individuals, such as astronauts and those who are directly exposed to ionizing radiation. It is clear that many studies should be conducted in this regard to obtain definitive and clinically valid results.

Conclusion

In summary, our findings confirm this hypothesis that the extracted serum due to unknown release factors and its injection into other groups of animals can induce a radio-adaptive response. Since the mechanism of this phenomenon is not well known, further studies are recommended focusing on the mechanism(s) as well as on cellular and molecular changes in the target groups. It might be speculated that the processes occurring in the body of the mouse lead to a phenomenon that increases the resistance of the body to high doses of ionizing radiation.

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