Look Different: Effect of Radiation Hormesis on the Survival Rate of Immunosuppressed Mice

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ABSTRACT

Background: Hormesis is defined as the bio-positive response of something which is bio-negative in high doses. In the present study, the effect of radiation hormesis was evaluated on the survival rate of immunosuppressed BALB/c mice by Cyclosporine A.

Material and Methods: We used 75 consanguine, male, BALB/c mice in this experiment. The first group received Technetium-99m (3700Bq) and the second group was placed on a sample radioactive soil of Ramsar region (800Bq) for 20 days. The third group was exposed to X-rays (3600Bq) and the fourth group was placed on the radioactive soil and then injected Technetium-99m. The last group was the sham irradiated control group. Finally, 30mg Cyclosporine A as the immunosuppressive agent was orally administered to all mice 48 hours after receiving X-rays and Technetium-99m. The mean survival rate of mice in each group was estimated during time.

Results: A log rank test was run to determine if there were differences in the survival distribution for different groups and related treatments. According to the results, the survival rate of all pre-irradiated groups was more than the sham irradiated control group ($p < .05$). The highest survival time was related to the mice which were placed on the radioactive soil of Ramsar region for 20 days and then injected Technetium-99m.

Conclusion: This study confirmed the presence of hormetic models and the enhancement of survival rate in immunosuppressed BALB/c mice as a consequence of low-dose irradiation. It is also revealed the positive synergetic radioadaptive response on survival rate of immunosuppressed animals.

Keywords

Radiation Hormesis, Survival Rate, BALB/c mice, Cyclosporine A, Radioadaptive Response

Introduction

It is about a century that human knows ionizing radiation is harmful for biological tissues and in acute doses it can lead to irreversible insult, cancer and even death. Moreover, various investigations have shown that the effect of low dose ionizing radiation is not predictable by extrapolating from damaging effects of high doses [1].

Hormesis is the stimulation of any system by low doses of any agent. It is defined as the bio-positive response of something which is bio-negative in high doses [2]. Although the origin of hormesis hypothesis was from pharmacological studies, various studies have shown that there may be hormetic effects in low dose ionizing radiation. Although the

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belief of "No-Safe-Level" is still widespread, this acclaim is proven by numerous positive biological effects of low dose ionizing radiation that are reported in hormetic or stimulatory models [3]. Recently, many scientists have clamored not to deprive people of bio-positive effects of low dose ionizing radiation and even proposed an annual single low dose ionizing radiation for everyone [4-7].

In the 1960s, a group of French researchers showed that parasites, protozoa and bacteria which received ionizing radiation below the normal background level, had a reduced reproduction rate. Their study disclosed that ionizing radiation was essential for the survival of those microorganisms [8].

In a research on mice, the incidence of leukemia, sarcoma and other cancers in irradiated mice with Cesium-137 at dose of 2.5-20 mSv was lower than the non-irradiated control group. The total number of malignancies in animals which were irradiated at dose of 10 mSv was approximately 30% lower than the non-irradiated group. Other studies also demonstrated the increased survival of irradiated animals at dose of 250-3000 mSv [9].

Ionizing radiation and a complex of other DNA damaging factors such as UV rays, alkylating agents, oxidants and heat shock can induce responses in order to recompense the initial insult. The obtained results of expand international studies specified that when cells were exposed to low dose of ionizing radiation and other DNA damaging agents, they showed higher resistance to high dose of the same and in some cases similar factors [10].

Adaptive response theory was reported for the first time in 1977 by Samson and Cairns. After discovery of this important phenomenon, it was determined that ionizing radiation could also induce this effect. In 1984, Olivieri et al. showed that exposure of human lymphocytes to labelled thymidine with tritium resulted in resistance against cellular damages due to high dose of X-rays. Their results known as "Radio-Adaptive Response" was very noteworthy since they represented that the radio-adaptive response reduced chromosomal aberrations in lymphocytes by roughly 50% [11].

Till now, numerous reports on the possible mechanism of the occurrence of adaptive effects and its factors have been published all over the world. In Iran, Mortazavi et al. have published several reports about the importance of radio-adaptive response, its effect on protection against radiation, role of natural radiation in occurrence of this effect and especially the incidence of radio-adaptive response in residence of high natural radiation areas of Ramsar County, Mazandaran Province in Iran [12-21].

As far as we know, the only study on the probability of radio-adaptive response using diagnostic doses of routine radioisotopes of the nuclear medicine was a research on patients who received I-131 [22]. The aim of the present study was to determine the quantity of radio-adaptive response and its effect on survival rate of immunosuppressed BALB/c mice by diagnostic dose of Tc99m, X-rays and a sample soil of a high level background radiation region (Ramsar County, Mazandaran Province, Iran). We also evaluated the effect of synergetic radioadaptive response on the survival rate of BALB/c mice subsequently receiving the $\frac{1}{2}$ median lethal dose (LD50) of Cyclosporine A.

Material and Methods

Study Population

In this experimental investigation carried out in the center of experimental animals of Shiraz University of Medical Sciences, we used 75 consanguine, male, BALB/c mice weighing 20-30 gram. Mice were purchased from Stem Cell and Transgenic Technology Research Center of Shiraz University of Medical Sciences, Iran. They were kept in a room with a temperature of between 22 and 24˚C and constant humidity in a cycle of 12 hour light and 12 hour dark. Adequate water and food was

provided. The room was disinfected before the study began by Sterl-STAT.

Experimental Design

The ethics committee of SUMS approved the study. Mice were randomly divided into 5 equal groups (15 mice in each group) and were coded. Decoding was done at the end of the study for bias prevention. All mice were maintained under the conditions described before. According to SUMS ethical codes regarding the care and use of laboratory animals, all possible steps were taken to avoid animal suffering at each stage of the experiment.

In the first group, each mouse received 0.5 ml (according to their weight) of Technetium-99m (3700 Bq radiation) via intraperitoneal injection. This quantity (dose/kg) of Technetium-99m is equivalent to injected dose/kg in human for bone scan. The radiotracer was injected in the hot lab of the nuclear medicine ward with a proper rate and under the supervision of a nuclear medicine specialist.

The second group was placed on a sample radioactive soil of Ramsar region (with approximately 800 Bq radiation) for 20 days. The amount of the sample soil was almost 2kg and brought from "Dasht-e-Sefid of Ramsar" region, Mazandaran Province in Iran (an area with a high level background radiation according to the declaration of international references and Iran Atomic Energy Agency).

The third group received an exposure of 70 kVp and 20 mAs X-rays (3600 Bq radiation) by a standard calibrated diagnostic instrument of radiology (Varian, Inc.).

The fourth group was placed on radioactive soil of Ramsar region for 20 days (800 Bq radiation) and then received 0.5 ml Technetium-99m (3700 Bq radiation).

The last group was the sham-irradiated control group with normal conditions. To avoid any bias, normal background radiation level soil was used in the cage of the control group and normal saline was injected to all groups without radiotracer injection.

The dose of Cyclosporine A was calculated according to body weight and with viewpoint of a pharmacologist. The oral median lethal dose (LD50) of Cyclosporine A in mice is 2329 mg/kg. A single dose of 1⁄2 Oral LD50 of Cyclosporine A was chosen to administer in order to suppress the immune system of animals entirely. Since the mean body weight of mice was estimated 25.8±4.35 gr, 30 mg Cyclosporine A was orally administered to all groups 48 hours after receiving Technetium-99m and X-rays. Consequently, 7 hours after the injection, monitoring of all groups started. Every 12 hours, the number of dead and living mice was counted in all groups.

Statistical Analysis

The gathered data were analyzed by SPSS V.22 software. The Kaplan-Meier method was used for survival analysis and comparison of the groups was done by Chi-Square nonparametric test. In all cases, p value < 0.05 was considered statistically significant.

Results

The number of dead and living mice at each count was compared with other groups. After 31 hours, all mice in the sham irradiated control group were dead. The mean survival time for the members of the control group was estimated 18.2±2.475 hours (Table 1).

A log rank test was run to determine if there were differences in the survival distribution for different groups and related treatments: Technetium-99m, soil of Ramsar region, X-ray, combination of soil of Ramsar region plus Technetium-99m and sham-irradiated control. The survival distributions for the five groups were statistically significantly different, $\chi^2(4) = 53.685$, $p < 0.0005$.

Based on the results, the general survival time of all pre-irradiated groups was more than the control group ($p < 0.05$) (Figure 1). The estimated mean time until death was only 18.2±2.475 hours for the control group. However, this measure was considerably more for

Table 1: Calculated Means and Medians for Survival Time of Different Groups

Treatment	Mean				Median			
			95% Confidence Interval				95% Confidence Interval	
	Estimate	Std. Error	Lower Bound	Upper Bound	Estimate	Std. Error	Lower Bound	Upper Bound
Technetium-99m	46.200	4.752	36.886	55.514	43.000	5.692	31.843	54.157
Soil of Ramsar	37.400	2.836	31.840	42.960	43.000	2.872	37.370	48.630
X-ray	31.800	2.978	25.963	37.637	31.000	4.554	22.075	39.925
Technetium-99m and Soil of Ramsar	61.400	5.476	50.667	72.133	67.000	7.303	52.686	81.314
Control	18.200	2.475	13.349	23.051	19.000	3.425	12.286	25.714

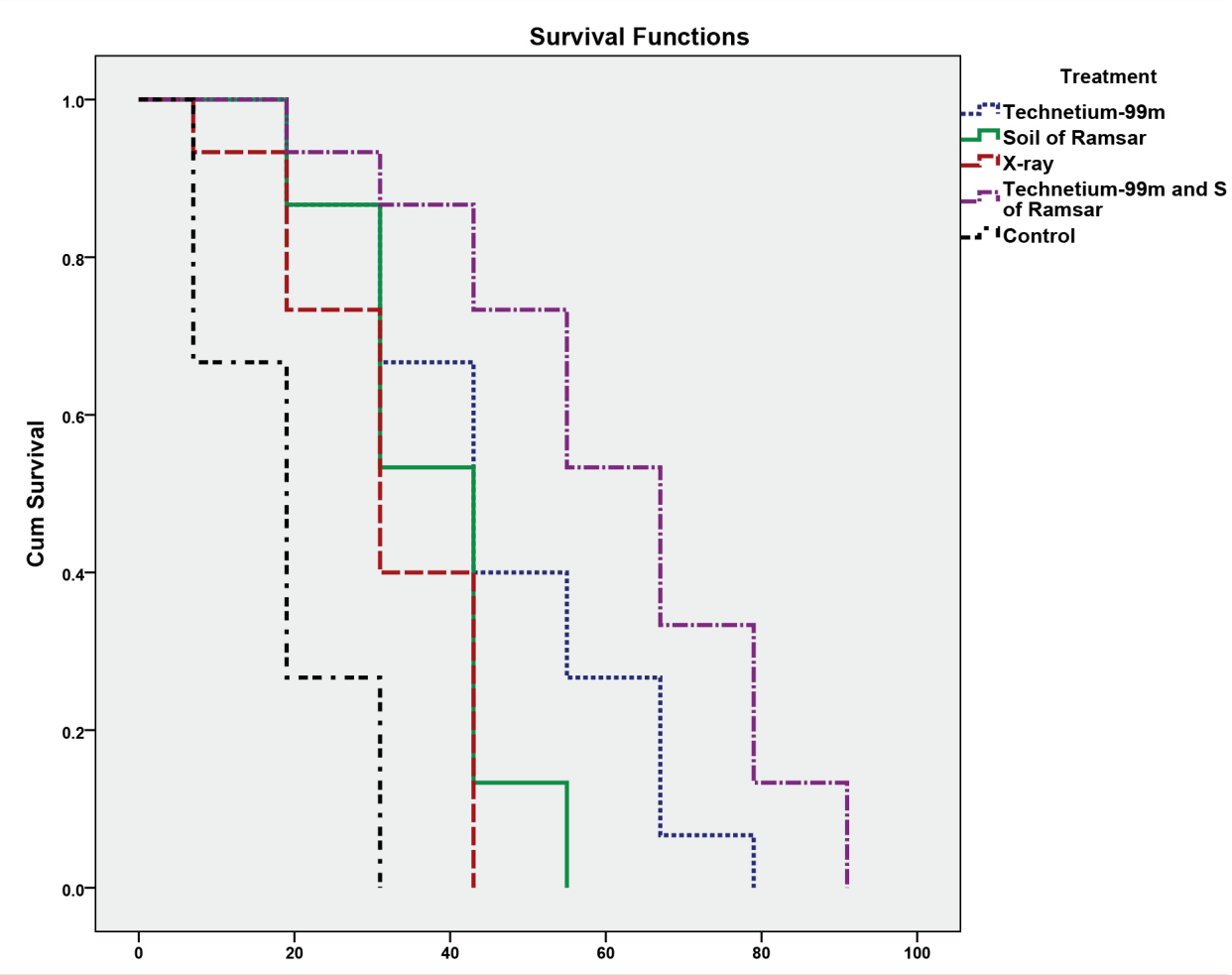

Figure 1: Survival Chart related to Different Treatments

pre-irradiated mice by receiving Technetium-99m (1st group) and combination of placing on the soil of Ramsar region plus receiving Technetium-99m (4th group), 46.2±4.752 hours ($p < 0.0005$) and 61.4±5.476 hours ($p < 0.0005$), respectively. The calculated survival time for the 2nd group (placing on the soil of Ramsar region) and the 4th group (pre-irradiated with X-rays) was also more than control group, 37.4±2.836 hours ($p < 0.0005$) and 31.8±2.978 hours ($p = 0.002$), respectively (Table 2).

Statistically, the survival rate of mice placed on the sample soil of Ramsar region for 20 days and then injected Technetium-99m 48 hours before the consumption of toxic dose of Cyclosporine A was significantly higher compared to other pre-irradiated groups ($p < 0.05$). According to the results, no significant difference was observed between pre-irradiated groups with X-rays and soil of Ramsar region ($p = 0.193$). Likewise, there was no significant difference in mean survival time of mice that received Technetium-99m and were placed on the sample soil of Ramsar region separately ($p = 0.073$). However, the survival time in the Technetium-99m group was profoundly more than the X-ray group ($p = 0.014$) (Table 2).

Discussion

Over the years, extensive concentration has generated to conduct research on protective and/or radio-resistance aspects of radio-adaptive response. In this context, an attempt was made in the present study to evaluate the efficacy of low-dose radiation in raising the survival rate of the immunosuppressed mice due to consumption toxic dose of Cyclosporine A.

Our findings revealed that all pre-irradiated groups represented a significantly higher resistance to immunosuppression and consequently death owing to consumption toxic dose of Cyclosporine A in comparison with the sham-irradiated control group ($p < 0.05$). Moreover, the highest survival time was related to the mice which were placed on the sample soil of Ramsar region (800 Bq) for 20 days and then injected Technetium-99m (3700 Bq) 48 hours before the consumption of toxic dose of Cyclosporine A so that the findings proved the considerable synergetic effect of the sample soil of Ramsar region and Technetium-99m combination on increasing the survival rate in immunosuppressed mice.

The first study in the field of adaptive response was conducted in 1977. Samson and Cairns reported that whenever cells were exposed to a low dose of mutant agents, repairing processes were induced in cells [10]. In 1996, Yonezawa et al. observed that when ICR-mice were pre-irradiated with 0.05 Gy of X-rays and then exposed to an 8 Gy radiation two months later, the 30-day survival rates of

Table 2: Pairwise Comparison Analysis between Groups

	Treatment	Technetium-99m		Soil of Ramsar		X-ray		Technetium-99m and Soil of Ramsar		Control	
		Chi-Square	Sig.	Chi-Square	Sig.	Chi-Square	Sig.	Chi-Square	Sig.	Chi-Square	Sig.
Log Rank (Mantel-Cox)	Technetium-99m			3.219	0.073	5.991	0.014	4.541	0.033	18.162	0.000
	Soil of Ramsar	3.219	0.073			1.696	0.193	12.936	0.000	15.886	0.000
	X-ray	5.991	0.014	1.696	0.193			16.268	0.000	10.000	0.002
	Technetium-99m and Soil of Ramsar	4.541	0.033	12.936	0.000	16.268	0.000			24.602	0.000
	Control	18.162	0.000	15.886	0.000	10.000	0.002	24.602	0.000		

pre-irradiated group was approximately 30% more than sham-irradiated group [23].

Bhattarcharjee in 1996 inspected that gamma irradiation of male BALB/c mice by a challenge dose of 2 Gy of Cobalt-60 led to the induction of thymic lymphoma (TL) in 46% of animals. Moreover, when the mice pre-irradiated with multiple adapting low doses of 1 cGy/day for 5 days (without a challenge dose), thymic lymphoma was induced in 16% of animals. Interestingly, when pre-irradiated mice were exposed to the challenge dose (2 Gy), thymic lymphoma was induced only in 16% of animals. Therefore, it seemed that low dose ionizing radiation (1 cGy) had a protective effect against the consequent high dose (2 Gy) to terminate the induction of thymic lymphoma in mice [24].

Mortazavi et al. in 2002 evaluated the adaptive response of long-term exposure to high-level background radiation in Ramsar region, Iran. Accordingly, they compared the blood samples of residence in the Ramsar region with those in an adjacent region that had a normal level background radiation. The frequency of chromosomal aberrations in lymphocytes was significantly lower in people living in region with high background compared to those in normal background areas consequently the administration of an in vitro challenge dose of 1.5 Gy of gamma rays to the lymphocytes. Moreover, they found that the frequency of chromosomal anomalies among Ramsar residence was statistically significantly lower than the control group who were living in a region with normal background radiation level. In addition, in 2003, they indicated that residence in regions with high level of background radiation had a greater resistance to radiation [12, 13, 15-21].

The formation mechanism of the adaptive response is still unknown. One of the best hypotheses about the mechanism of the adaptive response is induced repair processes of chromosomal aberrations owing to low-dose irradiation so that the damage of the later high dose irradiation will decrease. According to this theory, low doses of ionizing radiation induce the production of special proteins which are involved in DNA repair processes. Studies using two-dimensional gel electrophoresis indicated new proteins in cells irradiated with low doses of radiation [25-27].

In 1987, Feinendengen and his co-workers indicated that low doses of ionizing radiation caused a temporary inhibition in DNA synthesis. This temporary inhibition of DNA synthesis would provide a longer time for irradiated cells to recover. This inhibition also may induce the production of free radical scavengers, so irradiated cells would be more resistant to any further exposures [28].

Despite the fact that high doses of ionizing radiation are immunosuppressive, many studies have indicated that low dose radiation may stimulate the function of the immune system. In 1909, Russ first showed that mice treated with low-level radiation were more resistant against bacterial disease [29]. Later in 1982, Luckey published a large collection of references supporting immunostimulatory effects of low doses of ionizing radiation [30].

Similarly, our findings in this study confirmed the presence of hormetic models and the enhancement of survival rate among immunosuppressed animals because of low-dose irradiation. It is believed that studies such as this leading to the phenomenon become more apparent.

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Conflict of Interest

The authors report no declaration of interest.

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