The effect of whole body gamma irradiation on nitric oxide pathway of rat’s aorta
Ali Alavian-Ghavanini1,2*, Aminollah Bahaodini3, Ehsan Salimi2,4

Abstract
Introduction: Studies show that ionizing radiation impairs the so called blood vessels relaxation through inhibition of iNOS transcription without causing any morphological changes in them. This effect has been proven in Low Dose and Whole body irradiation. Thus this study focuses on the effect of chronic whole body irradiation on susceptibility of blood vessels to nitric oxide.

Methods: Twenty five adult male Wistar rats (weight 200 to 250 g) were divided into 5 groups, each allocated to chronic 6 days whole body cumulative doses of 0.84, 1, 1.5, 2, 2.5 gray using 137Cs as gamma ray source (dose rate of 0.014574 Gy/min). experiments performed shortly after 6 days irradiation by anaesthetizing rats using intraperitoneal injection of sodium pentobarbital (30 mg/kg) and excising thoracic aorta and immediately placing them in 4 ºC Krebs’ bicarbonate buffer. Each aortic ring was cut into 2-3 mm segments free from adherent connective tissue. The rings were suspended horizontally between two stirrups in organ chambers filled with 25 mL Krebs’ solution at 37 ºC and aerated continuously with 95% O2 and 5% CO2. One stirrup was connected to an anchor and the other was connected to a force transducer for recording isometric tension the mechanical responses of the segments. The data from 1st, 5th, 10th and 15th minutes was analyzed using ANOVA and Duncan at p≤ 0.05 as significance level.

Results were expressed as Mean ± SD.

Results: Results showed no significance differences between responses of isolated aorta strips.

Conclusions: It can be concluded that exposure of chronic whole body radiation may not influence the susceptibility of blood vessels to NO.

Keywords: Gamma irradiation, Nitric oxide, Aorta, Cardiovascular disease

Introduction
Men are all exposed to ionizing radiation from natural sources at all times. In this regard, the average background radiation to U.S. population has been reported to be up to 360 milirem (mrem) per year, which may vary mainly due to life conditions (1). The hazardous effect of whole body and/or partial body radiation has been always the subject of studies before and its harmful effect on physiological systems has been proved (2-4). Studies have also proved that ionizing radiation may defect vascular system through nitric oxide pathway causing cardiovascular complications (5-9). For example in a rat model it has been proved that a whole body 10 Gy ionizing radiation increases the risk of Coronary Sclerosis (8). It is proposed that the harmful effect of radiation in cardiovascular complications is through the impairment of endothelial dependent vasodilatation and specifically nitric oxide dependent relaxation. In a study on cervical arteries excited from the neck of patients who have been under radiation therapy, the impairment of nitric oxide relaxation through the impairment of eNOS transcription has been demonstrated (10). In a study performed by chines about the biological effect of 14 and 25 Gy radiation showed that the turnover of L-Arginine has been decreased, suggesting the augmentation of iNOS activity (11). It should be notified that the inconsistent results of studies mystify the link between radiation and production of nitric oxide; while a study shows that a 1 Gy chronic irradiation reduce the blood flow in coronary arteries in rat (12) another result shows the lack of any changes in nitric oxide dependent relaxation in rabbit arteries with a 6 Gy radiation dose (13).

The significant of studies about the effect of radiation on nitric oxide dependent relaxation lies on the fact that restenosis which is one of the most significant problems in post balloon angiography (10) has a treatment modality called “endovascular brachytherapy”. Although previous studies confirmed that radiation can hinder the occurrence of restenosis through stimulation of iNOS (14), nevertheless the consequence of different
Implication for health policy/practice/research/medical education

Previous studies showed that ionizing radiation impairs the so-called blood vessels relaxation through inhibition of iNOS transcription without causing any morphological changes. This effect has been proven in low dose and whole body irradiation. Hence this study, with the help of physiological data, will elucidate weather the whole body chronic radiation can change the sensitivity of blood vessels to nitric oxide. Clarification of this matter may therefore help other to better understand the effect of ionizing radiation on biological systems and through therapeutic interventions improve the treatment of cardiovascular disease associated with radiation exposure.

Materials and methods

Chemicals

L-NAME, acetylcholine, sodium pentobarbital and all the substances needed to form Krebs-Heinsleit solution were obtained from MERCK Company (MERCK, Germany). All drugs and substances were dissolved in distilled and de-ionized water and stocks were prepared for every experiment throughout the study.

Experimental Procedure

The study was performed on male wistar rats weighting 200 to 250 gram. They were treated according to the recommendation of internationally accepted principles for the care and use of experimental animals throughout the experiment. Groups of 5 animals were treated with chronic 6 days whole body irradiation and cumulative regimes of 0.8, 1, 1.5, 2, and 2.5 Gy using Cs-137 placed at Shiraz University's institute for radiation research as the gamma source with dose rate of 0.0147 Gy/min at 91.5 cm. All above regimes were proven to be capable in stimulating the production of nitric oxide and the linear genetic instability (14) and the radiation doses are well under the threshold for morphological changes in vascular tissues which is 8.8 to 8.3 Gy (15). The radiation treatments were given in a chronic 6 days period. During the radiation the animals were restrained in radiation field with a plexy box specifically designed for this study with 2 mm diameter. There were no changes in the food, water or caring of all the groups during the study and animals were closely observed for any unwanted changes with no such phenomenon was seen during the study.

Investigation was performed shortly after 6 days irradiation, according to previous studies, by anaesthetizing the rats via intraperitoneal injection of sodium pentobarbital (30 mg/kg) and excising thoracic aorta and preparing them in 2-3 mm wide. Afterward, aortic rings were freed from adherent connective tissue with strict attention to keep the endothelium intact. Aorta rings were mounted isometrically under a resting tension of 2 g in a flowing tissue bath, between a stationary stainless steel hook and an isometric force transducer coupled with a chart recorder. Vessels were maintained at 37 °C in an organ bath containing 25 ml of Krebs-Henseleit solution of the following composition (in mM): 118.3 NaCl, 4.7 KCl, 2.5 CaCl₂, 1.2 MgSO₄, 1.2 KH₂PO₄, 25.0 NaHCO₃, 11.0 glucose. This solution was continuously gassed with 95% O₂ and 5% CO₂. The ring was allowed to gain equilibrium for 45 min under resting tension before experiments commenced. Following equilibrium they were exposed to acetylcholine with concentration of 10⁻⁶ to 10⁻⁴ molar in cumulative manner two times and refilling the organ bath after each time. Again after washing the aortic ring and replacing the organ bath containing with new solution the aortic rings were exposed to L-NAME in concentration of 10⁻⁴ molar which has been previously treated with specific dosage of acetylcholine that its effect has been seen previously through the experiment, which in this case is 10⁻⁴ Molar. This method was repeated to make sure that the lowest statistical errors were obtained. The responses were recorded by bridge amplifier and chart 5 for windows software.

Statistical Analysis

Responses of each aortic ring to the specific concentrations of each drug which has been recorded by Chart 5 software were analyzed using the data analysis section of the software and were fished out in 1 minutes intervals. The data from 1st, 5th, 10th, 15th and 20th minutes were repeatedly analyzed using ANOVA and Duncan at p≤ 0.05 as significance level using SPSS version 17.0 (SPSS Inc., Chicago, IL, USA). Results were expressed as mean ± SD.

Results

Figure 1, illustrates a sample of the recorded tension; showing decrease in aortic ring tension following application of Ach.
Co-application of L-NAME, a nitric oxide synthase inhibitor, fully blocks the action of Ach indicating that reduction in tension is mediated by Nitric Oxide pathway. Figure 2, shows a limited dose response curve showing effects of cumulative concentrations of acetylcholine in control rats as well as rats treated with radiation. The y axis shows Normalized tension and the x axis shows minus logarithm of Ach concentrations. Tension decreases in a very similar way with incremental application of acetylcholine in all treatment groups as well as the control group. There were no statistical differences between the groups using ANOVA.

Figure 3, gives the effect of acetylcholine on normalized tension as well as their blockades by L-NAME. The y axis shows Normalized tension. It can be seen that, there was no statistically significant difference between the control and the group of rats treated with the highest dose of radiation. The blockade of acetylcholine effect by L-NAME, a nitric oxide synthase inhibitor, suggested that Nitric oxide pathway mediated the effects. This experiment was performed for all treatment groups and results were the same.

**Discussion**

It is generally known that acetylcholine which is a neurotransmitter and a stimulant to nitric oxide pathway causes relaxation in aortic rings suspended in the organ bath. L-NAME as the inhibitor of the NO pathway will cause contraction to suspended aortic rings in the same condition. Results showed a consistency with the theory as the tension has decreased after using acetylcholine and for the L-NAME the tension has increased after the decline caused by the usage of acetylcholine. Considering the previous studies, radiation brings about the impairment in function of blood vessels’ endothelial cells. Previously, it has been shown that ionizing radiation causes a decrease in endothelial dependent relaxation began with acetylcholine, although this effect has been seen only in concentration of acetylcholine above 100 nano-molars (16). Generally, it has been proven that radiation, like other stress substances e.g. HOCl, has harmful effect on vascular system through various mechanisms (6,17-19). For instance in 2009 Baker has proved that a total 10 Gy whole body radiation brings about complication in heart function and myocardial mechanism (8). Also in 2006, Suvorava et al. studied the dose rate effect of chronic and acute radiation on vascular system’s response. This study showed that, a 1 Gy chronic with the dose rate of $2.8 \times 10^{-7}$ Gymin$^{-1}$ during 41 days causes a decrease in coronary arteries’ blood pressure even after 90 days and also endothelial dependent relaxation has been suppressed after chronic irradiation and the level of suppression depends on dose rate and elapsed time after the experiment (12). Another study performed by Soloviev et al. on rabbit’s veins with a total 6 Gy radiation dosage and dose rate of 0.307 Gymin$^{-1}$, shows that even though the endothelia-dependent relaxation has not been impaired but the sensitivity of the veins to acetylcholine and L-NAME has been reduced (6).

Stochastic effects of radiation and various repairing mechanisms in biological systems such as adaptive response as well as wrong selection of dose rate are involved in the effect of radiation on biological systems; adaptive response and repairing mechanisms has an effective range between 0.02-0.05 Gy (20) and it has not been seen neither in all mammalian cells (21,22) nor in matured or dormant
cells (22). So it can be assumed that our dose rates were correctly adapted because the dose rate of 0.0145 Gy/min is well out of the range of adaptive response. Also it should be noticed that the matured vascular endothelial cells cannot be subjected to these repairing mechanisms. Also there were no threshold has been reported for impairment of nitric oxide pathway by radiation. There are some other studies showing the effect of different dose rates on biological systems. The effect of this dose rate was previously shown to be able to induce biological damages by Zaichkina et al. in 2004. They showed that, for chronic radiations with dose rate of 0.01 Gy/min the amount of cell damages were higher than expected (14). It also should not be ignored that in any other cases, non-targeted effects has still the capability of inducing radiation damages (22).

Also, there is a key difference in the sensitivity of different tissues and organs to radiation; one is the sensitivity of each cell alone and another is the tissue sensitivity as the aortic issues are considered sensitive to radiation (21,22). However, because no molecular investigation has been performed, there is no evidence to be convinced about the occurrence of these damages. We raised the question whether attenuation by the restrainer box was responsible for the lack of effect of radiation on nitric oxide pathway? To answer this question, we calculated the amount of attenuation caused by restrainer box using Monte Carlo code, namely MCNP4C simulation which is a recognized code for analyzing the transport and interaction of particles including gamma rays (23). Geometry parallel to the used radiation procedure was applied and photon flux was calculated 1 meter away from the source, in the presence and the absence of the restrainer (Figure 4). As can be seen in Table 1, the amount of attenuation was negligible. Hence, our observations were not confounded by restrainer box attenuation. Along these lines it can be concluded that the sensitivity of aortic rings and their response to acetylcholine and L-NAME and therefore the Nitric oxide pathway has not been changed due to irradiation of 0.8, 1, 1.5, 2, and 2.5 Gy with the dose rate of 0.0145 Gy/min\textsuperscript{-1}. Total radiation dose and radiation dose rate may be involved. Further physiological and molecular needed to be performed to establish an answer to the question of; whether the small whole body chronic radiation has harmful effect on human biological systems or not.

**Conclusions**

It can be concluded that chronic radiation did not affect NO-mediated relaxation of Wistar rat aorta. Mechanisms other than NO may be responsible for radiation induced vascular dysfunction. For example, an effect of radiation on various cell-repair mechanisms or physical effects of radiation on the cell could be responsible for these effects. Moreover, aortic NO pathway may be inherently less sensitive to radiation as opposed to medium and small arteries. A next step to these findings would be examining the effect of radiation on NO pathway in medium and small arteries.

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**Authors’ contributions**

All authors wrote the manuscript equally.

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**Ethical considerations**

Ethical issues (including plagiarism, data fabrication, double publication) have been completely observed by the author.

**References**


