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Short Communication

Evaluating the Protective Effect of a Combination of Curcumin and Selenium-L-Methionine on Radiation Induced Dual Oxidase Upregulation

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ABSTRACT

Background: Epidemiological studies have shown an increased incidence of heart diseases among survivors of Chernobyl disaster as well as Hiroshima and Nagasaki atomic bomb explosion. Similar results were observed for lung and left breast cancer patients. Experimental studies have proposed the chronic upregulation of some pro-inflammatory and pro-fibrotic cytokines. Recent studies have shown that upregulation of pro-oxidant enzymes play a key role in the development of late effects of ionizing radiation such as fibrosis. Interleukin-4 (IL-4) and Interleukin-13 (IL-13) are two important cytokines that have shown ability to induce production of free radicals through dual oxidases (Duox) i.e. Duox1 and Duox2. In this study, we aimed to detect the expression of IL-4 receptor-a1 (IL-4Ra1), IL-13 receptor-a2 (IL-13Ra2), Duox1 and Duox2 genes following irradiation of rat's heart. In addition, we evaluated the possible role of the combination of curcumin and selenium-L-methionine on the regulation of these genes.

Methods: Twenty rats were divided into 4 groups as follows; G1: control; G2: treatment with the combination of curcumin and selenium-L-methionine; G3: radiation; G4: radiation plus treatment with the combination of curcumin and selenium-L-methionine. Rats were sacrificed 10 weeks after irradiation for detecting the expression of IL-4Ra1, IL-13Ra2, Duox1 and Duox2.

Results: Results showed that exposure to ionizing radiation caused upregulation of IL-4Ra1 by more than 4-fold as well as Duox1 and Duox2 by more than 5-fold. However, results showed no detectable expression for IL-13Ra2. Treatment with the combination of curcumin and selenium-L-methionine could attenuate the upregulation of all genes.

Conclusion: This study has shown that exposing rat's heart tissues to radiation leads to chronic upregulation of IL-4Ra1, Duox1 and Duox2 as well as pro-oxidant enzymes. Treatment with the combination of curcumin and selenium-L-methionine showed ability to attenuate the upregulation of these genes.

Introduction

Heart diseases are among the most common indications of death in different populations. Evidences have shown that exposure to ionizing radiation is one of the reasons for the increasing incidence of heart diseases.[1] This has been confirmed for both clinical and accidental exposure to ionizing radiation.[2] Some studies showed increased heart diseases among patients with left breast cancer who had undergone radiotherapy.[3] This was also confirmed for patients who had been exposed to whole body irradiation for other indications such as hematopoietic stem cell transplantation.[4] In addition to clinical radiotherapy, increased incidence of heart disorders has been observed among survivors of Chernobyl disaster as well as Hiroshima and Nagasaki atomic bomb explosion.[5,6] Further analyses have shown increased disorders for carotid and coronary artery, impaired blood supply to heart muscles, pericarditis, increase in collagen

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deposition and increased serum level of total cholesterol and triglycerides that may lead to atherosclerosis.[7,8]

Emerging evidences from experimental studies have shown that changes in the secretion of cytokines play a key role in the development of cardiovascular disorders following exposure to radiation.[9] Exposure to a high dose of radiation leads to cell death through apoptosis, necrosis and mitotic catastrophe. Necrosis and apoptosis leads to infiltration of inflammatory cells such as mast cells, lymphocytes and macrophages. This leads to chronic secretion of pro-inflammatory and pro-fibrotic cytokines such as interleukins (IL) (IL-1, IL-4, IL-6, IL-8, IL-10), IL-13, Tumor necrosis factor-α (TNF-α), Transforming growth factor (TGF-β), etc.[10] Studies proposed that continuous production of free radicals through immune system-redox interactions play a key role in late effects of exposure to radiation in heart tissue, including inflammation and fibrosis.[11] For example, TGF-β is able to upregulate the expression of some pro-oxidant enzymes such as NADPH oxidase (NOX)-1, cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS).[12] Upregulation of these enzymes is involved in radiation-induced chronic oxidative stress and fibrosis. Their inhibition have been proposed for amelioration of these side effects.[13] Dual oxidase (Duox) genes such as Duox1 and Duox2 are two ROS producing enzymes that are involved in chronic oxidative stress during some stress conditions. A study by Ameziane et al. showed that upregulation of IL-4 and IL-13 following exposure to radiation induces the expression of Duox1 and Duox2, leading to chronic production of free radicals and genomic instability.[14]

In recent years, several studies have shown that natural antioxidants and flavonoids are able to protect against toxic effects of ionizing radiation.[15] Studies proposed that in addition to direct antioxidant role, modulation of immune responses and pro-oxidant genes play a key role in the radioprotective effect of flavonoids.[16] Curcumin is the most common flavonoid that has been studied for amelioration of radiation injury in different organs.[17] It has ability to target several pro-inflammation and pro-fibrotic mediators. In addition, it has an antioxidant effect that causes neutralization of free radicals.[18] On the other hand, selenium-L-methionine has a potent antioxidant effect due to the presence of both selenium and methionine. Both selenium and methionine can scavenge free radicals directly. Furthermore, selenium through stimulation of antioxidant enzymes such as glutathione (GSH) and superoxide dismutase (SOD) has an indirect antioxidant role.[19] In present study, we studied the effect of a combination of curcumin and selenium-L-methionine on the expression of Duox1 and Duox2 expression.

Materials and Methods

Drug treatment and irradiation

Curcumin and selenium-L-methionine were purchased from Sigma Aldrich (USA). A total of 20 male Wistar albino rats were procured from Razi institute, Tehran, Iran. Irradiation was performed using a cobalt-60 gamma ray source. RNX-Plus was purchased from Sinaclon (Iran) while cDNA synthesis kit was purchased from Takara (Japan).

Curcumin was dissolved in 20% ethanol while selenium-L-methionine was dissolved in distilled water. The concentration of curcumin was 30 mg/ml while that of selenium-L-methionine was 0.8 mg/ml. Each rat was treated orally with 1 ml curcumin (150 mg/kg) in addition to intraperitoneal administration of 1 ml selenium-L-methionine (4 mg/kg), 1 day before irradiation to 3 consecutive days after irradiation. On the day of irradiation, treatment was done 30 minutes before irradiation. To ensure proper fixation, all rats received an intraperitoneal injection of ketamine 10% (80 mg/kg) and xylazine 2% (5 mg/kg) as anesthesia. The chest area was irradiated using cobalt-60 gamma rays (1.25 Mev) at a dose rate of 109 cGy per minute.

Experimental design

Rats were divided into 4 groups namely 1: control (no irradiation or drug treatment); 2: drug treatment: rats received a combination of curcumin and selenium-L-methionine 1 day before and 3 consecutive days after irradiation; 3: irradiation: rats received 15 Gy gamma rays to chest area; 4: radiation plus drug treatment: rats received 15 Gy gamma rays as well as a combination of curcumin and selenium-L-methionine 1 day before and 3 consecutive days after irradiation. 10 weeks after irradiation, rats were sacrificed and their heart tissues collected. All tissues were frozen at -80°C. This study was approved by ethical committee of Tehran University of Medical Sciences.

RNA extraction and cDNA synthesis

At first, heart tissues were defrosted and homogenated in RNX-Plus at low temperature. Total RNA was obtained after homogenation and extraction using RNX-Plus according to manufacturer's instructions. Integrity and purification of extracted RNA was detected using electrophoresis ethidium bromide stained in agarose-Tris-borate ethylenediaminetetraacetic acid gels. Furthermore, the purity of extracted RNA was detected using a Nanodrop instrument which showed an absorbance ratio of more than 2 for A260 nm/A280 nm. After confirmation of extracted RNA, the cDNA was synthetized from RNA using cDNA synthesis kit. For cDNA synthesis, 1 µg of total RNA from each sample was used. The RNA was first denatured at 65°C for 10 minutes and then reverse transcribed at 45°C for 30 minutes using thermocycler.

Real-time PCR

The expression of genes was detected using Applied Biosystems real-time PCR devise (USA). GAPDH (glyceraldehyde-3-phosphate dehydrogenase) was chosen as internal control gene for housekeeping. For evaluating the expression of each gene, the specified primer was designed using Generunner (Version 3.05; Hastings software Inc., Hastings, USA) and then blasted in NCBI. The sequences of designed primers are shown in Table 1.

Initial denaturation of cDNA was carried out at 95°C for 2 minutes. Afterwards, 40 cycles was used for denaturation at 95°C for 15 seconds. Annealing and elongation was performed at 60°C for 15 seconds, and 72°C for 15 seconds respectively. For calculating relative mRNA levels, the real-time PCR efficiency for target and housekeeping was determined using the slope of a linear regression model.[20] For each sample, real-time PCR was performed as duplicate. The comparative method ($2^{-\Delta\Delta CT}$) which is different among ΔCT for control and treatment groups was used for calculating the relative changes in the expression of IL-4 receptor-a1 (IL-4Ra1), IL-13 receptor-a2 (IL-13Ra2), Duox1 and Duox2 compared to housekeeping gene.

Statistical Analysis

For statistical analysis, SPSS software version 24 was used. Test for significance between groups was evaluated using analysis of variance (ANOVA) as well as post HOC Tukey's HSD. P value <0.05 was considered statistically significant.

Table 1. The sequences of primers for Real Time PCR.

Gene	*Forward sequence*	*Reverse sequence*
IL-13Ra2	5′ TCGTGTTAGCGGATGGGGAT 3′	5′ GCCTGGAAGCCTGGATCTCTA 3′
Duox1	5′ AAGAAAGGAAGCATCAACACCC 3′	5′ ACCAGGGCAGTCAGGAAGAT 3′
IL-4R1	5′ GAGTGAGTGGAGTCCCAGCATC 3′	5′ GCTGAAGTAACAGGTCAGGC 3′
Duox2	5′ AGTCTCATTCCTCACCCGGA 3′	5′ GTAACACACACGATGTGGCG 3′
GAPDH	5′ AGTGCCAGCCTCGTCTCATA 3′	5′ ATGAAGGGGTCGTTGATGGC 3′

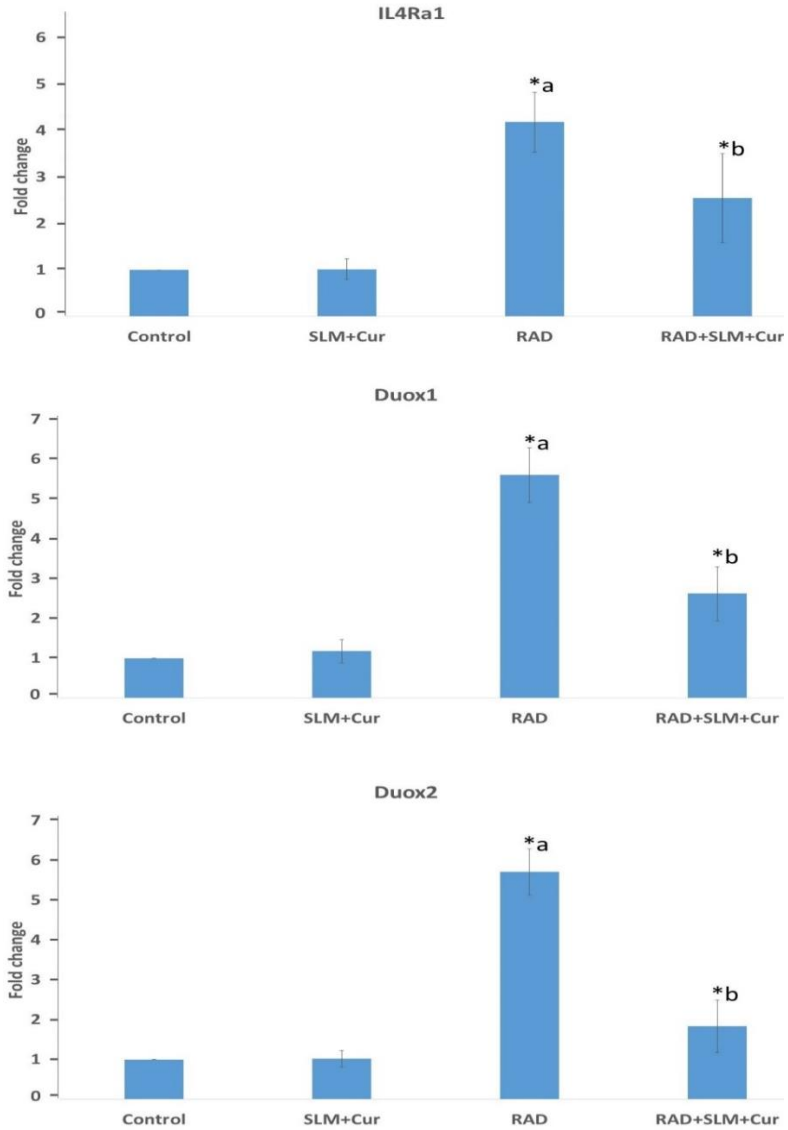

Figure 1. The results of expression of IL4Ra1, Duox1 and Duox2 after irradiation of rat's heart and treatment with the combination of curcumin and selenium-L-methionine. Results have expressed as mean ± standard deviation (SD). a: significant compared to control; b: significant compared to radiation group, with p values <0.05. SLM= selenium-L-methionine; Cur=Curcumin; RAD= Radiation.

Results

Results (Figure 1) showed that irradiation of heart tissues caused significant upregulation of IL4Ra1 (4.19±0.65 fold) ($p<0.05$). Treatment with the combination of curcumin and selenium-L-methionine before and after exposure to radiation could attenuate the expression of IL4Ra1 (2.55±0.95 fold) ($p<0.05$). However, in rats treated with the combination of curcumin and selenium-L-methionine without irradiation, there was no change in the expression of IL4Ra1 (1.02±0.22 fold). We did not detect any expression of IL13Ra2. Results of Duox1 gene expression showed that irradiation caused significant increase in its expression compared to control group (5.60±0.69 fold) ($p<0.05$). Treatment with the combination of curcumin and selenium-L-methionine reduced the expression of Duox1 in comparison to radiation group (2.62±0.68 fold) ($p<0.05$). Treatment with the combination of curcumin and selenium-L-methionine without irradiation did not change the expression of Duox1 compared to control group (1.18±0.30 fold). Results of Duox2 gene expression showed a significant increase in its expression after irradiation of rat's chest (5.71±0.58) ($p<0.05$). Treatment with the combination of curcumin and selenium-L-methionine before and after irradiation reduced Duox2 gene expression (1.84±0.66) ($p<0.05$). However, rats treated with the combination of curcumin and selenium-L-methionine without irradiation did not show any significant change in the expression of Duox2 (1.02±0.21 fold).

Discussion

As our results have shown, exposing rat's heart tissues to an acute dose of gamma rays leads to significant increase in the expression of IL4Ra1, Duox1 and Duox2. However, results of this study showed no detectable expression of IL13Ra2. Treatment with the combination of curcumin and selenium-L-methionine without irradiation did not change the expression of either genes compared to control group. However, when rats were treated with the combination of curcumin and selenium-L-methionine before and after irradiation, the expression of all genes reduced significantly. The most obvious changes were detected for Duox1 by more than 5-fold increase following irradiation and reduced to lower than 2-fold following treatment. Results indicated that exposing heart tissues to ionizing radiation may lead to an increase in IL-4, a pivotal stimulator of fibrosis. In addition, our results have shown that the combination of curcumin and selenium-L-methionine may reduce the risks of heart fibrosis through suppression of IL-4Ra1 gene expression. However, there is a need for further studies using histological evaluation to confirm these findings.

These results have given an indication that upregulation of dual oxidase gene (Duox1 and Duox2) expression may be involved in chronic oxidative stress after exposure to ionizing radiation. A study by Hassani et al. showed that exposing thyroid cells to ionizing radiation causes chronic upregulation of Duox1, thereby facilitating the production of ROS and DNA damage. This study has shown that IL-13 is mainly responsible for stimulating Duox1, whose effect is mediated through the main receptor of IL-4 and IL4Ra1.[14] Results of our study also showed that the increased expression of these pro-oxidant genes can be attenuated using a combination of curcumin and selenium-L-methionine. Previous studies have revealed that exposure to a high dose of radiation leads to massive DNA damage and cell death through stimulation of necrosis and apoptosis. As earlier mentioned, cell death following exposure to radiation leads to the release of a heavy amount of both inflammatory and tolerogenic cytokines which are able to induce several signaling pathways including pro-oxidant enzymes. Free radical production, inflammatory mediators and pro-oxidant enzymes amplify the activities of each other in a positive feedback loop. Suppressing the activities of pro-oxidant enzymes and inflammation has been proposed for mitigation of radiation injury.

Previous studies have shown that curcumin has ability to reduce radiation responses through modulation of several immune system mediators such as NF-kB, STATs, AP-1, etc.[21,22] Furthermore, curcumin has ability to attenuate oxidative stress and activities of pro-oxidant enzymes like COX-2.[23] Curcumin has also shown ability to reduce micronuclei formation after whole body irradiation.[24] In addition, it reduces free radical production as well as oxidative injury.[25] Through inhibition of inflammatory cytokines, curcumin ameliorates radiation toxicity in some organs such as lung, skin and gastrointestinal system.[26-28] On the other hand, selenium-L-methionine has shown ability to attenuate redox interactions as well as chronic production of free radicals.[29-31] The main effect of selenium-L-methionine is related to stimulation of antioxidant enzymes. [19] It has shown ability to attenuate endogenous production of free radicals and reduce radiation toxicity in highly radiosensitive organs such as bone marrow, gastrointestinal system and kidney.[30,31] The combination of curcumin and selenium-L-methionine may be useful for attenuation of both inflammation and redox activity, which in turn may reduce radiation injury more effectively.[24]

Conclusion

This study showed that exposing rat's heart tissues to ionizing radiation leads to chronic upregulation of Duox1 and Duox2, two important pro-oxidant enzymes. IL-4 and its receptor IL4Ra1 play a key role in the regulation of these genes. Treatment with a safe dose of the combination of curcumin and selenium-L-methionine 1 day before to 3 consecutive days after irradiation showed ability to attenuate the upregulation of IL4Ra1, Duox1 and Duox2. This combination is able to reduce radiation injury through modulation of redox system and chronic oxidative stress.

Conflict of interests

The authors claim that there is no conflict of interest.

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