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Radioprotective effects of selenium and vitamin-E against 6MV X-rays in human blood lymphocytes by micronucleus assay

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Abstract

Background: Critical macromolecules of cells such as DNA are in exposure to damage of free radicals that induced from the interaction of ionizing radiation with biological systems. Selenium and vitamin-E are natural compounds that have been shown to be a direct free radical scavenger. The aim of this study was to investigate the radioprotective effect of selenium and vitamin-E separately and synergistically against genotoxicity induced by 6MV x-rays irradiation in blood lymphocytes.

Methods: Fifteen volunteers were divided into three groups include A, B and C. These groups were given selenium (800IU), vitamin-E (100mg) and selenium (400IU) + vitamin-E (50mg), respectively. Peripheral blood samples were collected from each group before (0hr) and 1, 2 and 3hr after selenium and vitamin-E administration (separately and synergistically). Then the blood samples were irradiated to 200cGy of 6MV x-rays. After that lymphocyte samples were cultured with mitogenic stimulation to determine the chromosomal aberrations with micronucleus assay in cytokinesis-blocked binucleated cells.

Results: The lymphocytes in the blood samples collected at one hr after ingestion selenium and vitamin-E, exposed in vitro to x-rays exhibited a significant decrease in the incidence of micronuclei, compared with control group at 0hr. The maximum protection and decrease in frequency of micronuclei (50%) were observed at one hr after administration of selenium and vitamin-E synergistically.

Conclusion: The data suggest that ingestion of selenium and vitamin-E as a radioprotector substance before exposures may reduce genetic damage caused by x-rays irradiation.

Keywords: 6MV, X-rays, Selenium, Vitamin-E, Lymphocyte, Micronucli.

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Introduction

Radiotherapy with ionizing radiation could damage the human tissues (1). DNA (genetic material) in the nucleus and mitochondria of cells, recognized is the most critical target of ionizing radiation (2). Ionizing radiation generates reactive oxygen species (ROS) in the radiation-exposed cells, these free radicals such as hydroxyl (˙OH) and (˙H) can induce damage to critical macromolecules such as DNA (3). DNA damage may cause mutations and cancer (3). Since radiation-induced cellular damage is attributed primarily to the harmful effects of free radicals, molecules with radical scavenging properties are particularly promising as a radioprotector (4). Selenium and vitamin-E are two famous radioprotectors that much research has been done on them (5,6). Selenium is a trace element that is naturally present in many foods and available as a dietary supplements (5,7). Selenium has been shown to be a direct free radical scavenger and an indirect antioxidant via its stimulatory actions on antioxidant enzymes

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activity and inhibitory actions on pro-oxidative enzymes activity (7). Many studies indicated selenium antioxidant properties can effects on DNA repair, apoptosis, and the endocrine and immune systems, Moreover selenium might play a role in the prevention of cancer (5,8-10).

Vitamin-E is the collective name for a group of fat-soluble compounds with distinctive antioxidant activities. It is found naturally in some foods and available as dietary supplements (11). Antioxidant nutrients like vitamin-E protect cell constituents from the damaging effects of free radicals that if unchecked might contribute to cancer development (6).

For assessing chromosome damage in this study, micronuclei test was used. A micronucleus is a test that is used in toxicological screening for potential genotoxic compounds. This assay is now recognized as one of the most successful and reliable assays for genotoxic carcinogens, i.e., carcinogens that act by causing genetic damage (12). In this analyze, the cytochinesis is blocked and any numerical or structural damages to chromosomes would be viewed as minute nuclei in binucleate cells called micronucleus (MnBi). Micronuclei (Mn) may originate from either acentric chromosomal fragments or whole chromosome delayed in anaphase. This assay has being used widely to investigate the effects of different probable radioprotectors (12,13).

Although many studies showed that treatment of human peripheral blood lymphocytes in vitro with selenium and vitamin-E reduce micronuclei induced by gamma irradiation, the efficiency of these substances has been not evaluated in vivo by oral administration. Here, we evaluate the efficacy of selenium and vitamin-E separately and synergistically in human volunteer's blood lymphocytes with a method combining in vivo exposure of the volunteers to the selenium and vitamin-E and in vitro testing of the radioprotective effect against 6MV x-rays

Methods

Data & Design

This study was performed after obtaining permission from the medical ethics committee of the Tehran University of Medical Science. Clinical trial code of this study is IRCT2015090123843N1. In this study fifteen healthy, non-smoking male volunteers (mean±SD age of 26±3yrs) with an average weight of 69 kg, whom had no medicines or drugs taken for at least two months prior to sampling and not suffering from any known serious acute or chronic illness, were selected and informed consent was obtained from them. These volunteers divided into three groups include A, B and C. After of overnight fasting, five volunteers (A group) were given one gelatin capsules containing 800 IU Selenium, five volunteers (B group) were given capsule containing 100 mg of vitamin-E and five volunteers were given vitamin-E plus selenium gelatin capsule(containing 400 IU vitamin E and 50 mg selenium) at 8 am. All vitamin-E and selenium gelatin capsules contain standard dose (FDA approve) that were purchased from American natural company. Then blood samples were collected in heparinized tubes 10 min before (0 hr) and 1, 2, and 3 hr after selenium (A group), Vitamin-E (B group) and selenium plus vitamin-E (C group) ingestion.

At each sampling time, for each volunteer, 1ml aliquots of heparinized whole blood was divided into two 25 ml culture flasks. One flask was the non-irradiated control sample, and another was irradiated at 37°C (temperature) and 87.9 kPa (pressure) with 6Mv x-rays accelerator (Oncor, Germany Siemens) with a dose of 200 cGy at a 100cm SSD and 10 cm^2 field size. In order to provide uniformity of dose distribution throughout the whole sample, two opposite x-rays beams were directed perpendicular to the longer axis of the flask containing the blood sampels. In each of these two fractions, half of the planned dose was delivered (14).

Subsequently, 0.5ml of each samples (non-irradiated control and irradiated) was

added to 4.5ml of RPMI 1640 culture medium (Sigma, U.S), which contained a mixture of 20% fetal calf serum, 100µl/ml phytohaemagglutinin (Sigma, U.S), 100µl/ml penicillin, 250µg/ml streptomycin and 2mM glutamine (Sigma, U.S) at final concentration. All cultures were incubated at 37±1°C in a humidified atmosphere of 5% CO_2 and 95% O_2. Cytochalasin B (Sigma-Aldrich, final concentration: 6µg/ml) was added after 44 hr of culture (13). After 72 hr of incubation, the cells were collected by centrifugation for 5 min at 800r.p.m, resuspended in 0.075M cold potassium chloride for 6 min at 800r.p.m and immediately fixed in a fixative solution(methanol: acetic acid,6:1) three times. The fixed cells were dropped onto clean microscopic slides, air-dried and stained with Giemsa solution. All slides were evaluated at 1000× magnification to determine the frequency of micronuclei in cytokinesis-blocked binucleate cells with the well preserved cytoplasm. Small nuclei were scored as micronuclei if their diameters were between 1/16 to 1/3 of main nuclei and were non-refractile, not linked to main nuclei and not overlapping the main nuclei (13). At each blood collection time and for each volunteer, 1000cells were examined from the irradiated and control cultures in duplicate to record the frequency of micronuclei.

Statistical Analysis

The t-test was used to compare the incidence of micronuclei in each time point (1, 2, 3 hr) with the 0 hr time irradiated control points. The level of statistical significance was set at $p<0.05$.

Results

There were no side effects observed after the single oral dose ingestion of selenium and vitamin E in all five volunteers at 0 hr (before selenium and vitamin E administration). The obtained data (Table 1, 2 and 3) showed a significant increase in the incidence of micronuclei in x-rays irradiated lymphocytes in compare to the control cells without irradiation. A typical micronucleated binucleates is shown in Figure 1.

The percentage of micronuclei induced by irradiation in lymphocytes of all volunteers at 1, 2 and 3 hr after eating of selenium alone (A group) was 6.08±0.22, 7.74±0.34 and 9.32±0.42, respectively. The percentage of micronuclei found in the selenium-treated cell groups were significantly much lower than the control point with radiation alone at 0 hr. Lymphocytes in the blood samples collected at 1 hr after the oral eating of materials (exposed in vitro to

Table 1. The percentage (%) of micronuclei induced in vitro by exposure to 2Gy of 6-MV-radiation in cultured human blood lymphocytes at 10 min before(0 hour) and at 1, 2 and 3 hours after oral ingestion of selenium (800 IU) alone ($p<0.05$).

Volunteer	At 0hour (10 min before ingestion)		At 1hour (1 hour after ingestion)		At 2hours (2 hours after ingestion)		At 3hours (3 hours after ingestion)	
	Control	Irradiated	Control	Irradiated	Control	Irradiated	Control	Irradiated
1	0.1	10.0	0.0	5.9	0.0	7.4	0.0	9.6
2	0.0	10.8	0.1	6.3	0.1	7.4	0.1	9.0
3	0.0	11.2	0.2	6.2	0.1	8.0	0.0	8.9
4	0.2	11.6	0.2	6.0	0.0	8.1	0.2	9.3
5	0.0	11.0	0.1	6.0	0.1	7.8	0.2	9.8

Table 2. The percentage (%) of micronuclei induced in vitro by exposure to 2 Gy of 6-MV-radiation in cultured human blood lymphocytes at 10 min before (0 hour) and at 1, 2 and 3 hours after oral ingestion of Vitamin-E (100mg) alone ($p<0.05$).

Volunteer	At 0hour (10 min before ingestion)		At 1hour (1 hour after ingestion)		At 2hours (2 hours after ingestion)		At 3hours (3 hours after ingestion)	
	Control	Irradiated	Control	Irradiated	Control	Irradiated	Control	Irradiated
1	0.1	10.8	0.0	6.2	0.0	8.0	0.0	10.0
2	0.0	10.8	0.1	6.3	0.0	7.8	0.1	9.8
3	0.0	11.0	0.2	6.5	0.1	8.4	0.2	9.2
4	0.2	11.4	0.2	7.0	0.2	8.6	0.2	9.8
5	0.0	11.3	0.1	6.8	0.1	8.2	00	9.9

Table 3. The percentage (%) of micronuclei induced in vitro by exposure to 2Gy of 6-MV-radiation in cultured human blood lymphocytes at 10 min before(0 hour), and at 1, 2 and 3 hours after oral ingestion of selenium(400 IU) plus vitamin-E (50mg) ($p<0.001$).

Volunteer	At 0hour (10 min before ingestion)		At 1hour (1 hour after ingestion)		At 2hours (2 hours after ingestion)		At 3hours (3 hours after ingestion)	
	Control	Irradiated	Control	Irradiated	Control	Irradiated	Control	Irradiated
1	0.0	10.4	0.0	4.8	0.0	6.1	0.0	6.6
2	0.0	10.7	0.1	5.3	0.0	6.4	0.1	6.8
3	0.3	11	0.2	5.9	0.1	6.5	0.2	6.9
4	0.2	11.1	0.2	6	0.2	6.7	0.2	7
5	0.1	10.8	0.1	5	0.1	6.3	00	6.7

2 Gy of 6 Mv radiation) exhibited significant decrease in the incidence of micronuclei as compared with similarly irradiated lymphocytes in the blood collected at 0 hr ($p<0.05$). The total micronuclei values were 45% fold less after one hr from the oral administration of selenium. Total micronuclei usually were lower at one hr as compared with those at 2 and 3 hr after the oral administration of selenium alone (Table 1).

The percentage of micronuclei induced by irradiation in lymphocytes of all volunteers at 1, 2 and 3 hr after eating of vitamin-E alone(B group) was 6.56±0.44, 8.2±0.4, and 9.74±0.26, respectively. The percentage of micronuclei found in the vitamin-E treated cell groups were significantly much lower than the control point with radiation alone at 0 hr. Lymphocytes in the blood samples collected at 1 hr after the oral eating of vitamin-E (exposed in vitro to 2 Gy of 6 Mv radiation) exhibited significant decrease in the incidence of micronuclei as compared with similarly irradiated lymphocytes in the blood collected at 0 hr ($p<0.05$). The total micronuclei values were 41% fold less after 1 hr from the oral administration of vitamin E. Total micronuclei usually were lower at 1 hr as compared with those at 2 and 3 hr after the oral administration of vitamin E alone (Table 2).

The percentage of micronuclei induced by irradiation in lymphocytes of all volunteers at 1, 2 and 3 hr after eating of selenium plus vitamin-E (C group) was 5.4±0.6, 6.4±0.3 and 6.8±0.3, respectively. The percentage of micronuclei found in the selenium and vitamin-E treated cell groups were significantly much lower than the control point with radiation alone at 0 hr. Lympho-

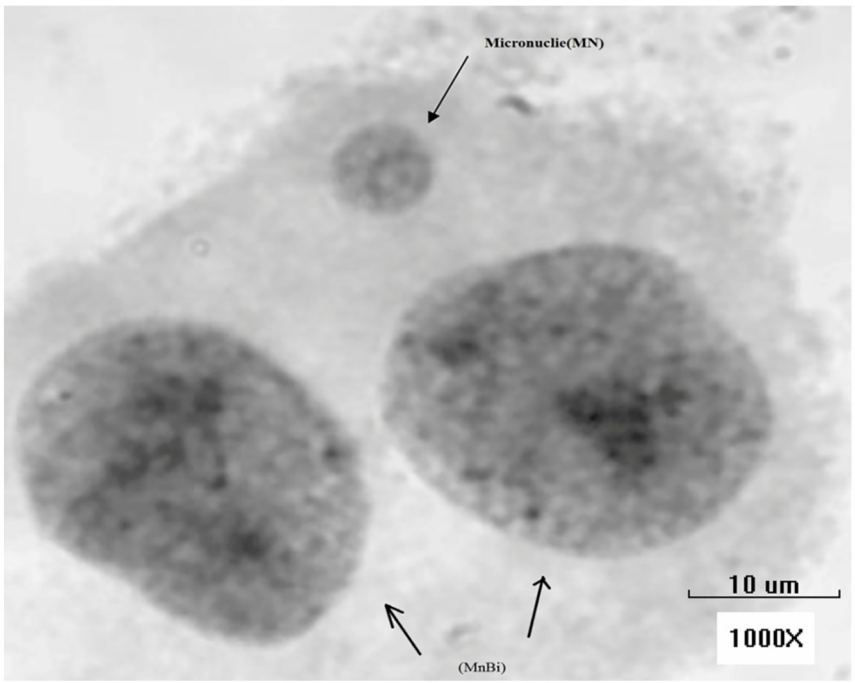

Fig.1. Typical micronucleated binucleate

cytes in the blood samples collected at 1 hr after the oral eating of selenium and vitamin-E (exposed in vitro to 2 Gy of 6 Mv radiation) exhibited significant decrease in the incidence of micronuclei as compared with similarly irradiated lymphocytes in the blood collected at 0 hr ($p<0.001$). The total micronuclei values were 50% fold less after 1 hr from the oral administration of selenium and vitamin E. Total micronuclei usually were lower at 1hr as compared with those at 2 and 3 hr after the oral administration of selenium and vitamin E synergistically (Table 3).

Differences between the total number of micronuclei recorded after treatments with selenium and vitamin-E in irradiated samples and not treatment irradiated samples were statistically significant ($p<0.05$).

Discussion

Radioprotectors with high performance can be used to protect people in cases like occupational exposures, nuclear accidents, radiotherapy and cosmic rays exposures (15-17).

Two-thirds of chromosomal damage induced by ionizing radiation are due to free radicals; it is regarded as the indirect effects of radiation (3). Compounds include antioxidant element reduce the effects of ionizing radiations in living systems such chromosomal damage due to scavenging free radicals (4). Many studies have demonstrated selenium and vitamin-E ability to scavenge free radicals (5,6,8, 10,11).

The data from Noaman et al. suggests the synergistic use of Vitamin-E and selenium which cause a greater reduction in dangerous enzymes such: glutathione (GSH), glutathione peroxidase (GSH-Px), and superoxide dismutase (SOD) in a blood sample of irradiated rats in compare separately use of vitamin E and selenium (19).

Moreover many studies have shown the radioprotective effect of selenium and vitamin-E separately, since both of them are natural and inexpensive compounds, however, few studies have been carried out on these two, according to human volunteers (6,7,9,18). This study confirmed the ability of x-rays -radiation to increase the incidence of MN in exposed human lymphocytes, as reported previously in many other investigations (2,3,18,20). The result of our study did not show any side effect from eating selenium and vitamin-E in human volunteers. High reduction about 50% in chromosomal abnormalities by exposure to 2Gy of 6-Mv-radiation in blood lymphocytes seen after administration of selenium and vitamin E synergistically. In vitro, similar studies on human blood lymphocytes also show a significant decrease in chromosomal damage in the presence of selenium and vitamin-E after irradiation of gamma rays (6,9,18).

In this study 2 Gy of 6Mv-radiation was selected because it is a standard dose fraction that is applied daily to cancer patients in fractionation regimes in radiotherapy and high sensitivity of human DNA is in 2 Gy radiation (21). The results of this study showed the greatest effect of radioprotection on blood lymphocytes (in all three groups) was one hour after administration of selenium and vitamin-E, and after that, this effect was reduced. The results of this study also showed that concomitant use of selenium and vitamin E have a more radioprotective effect than using these separately. There were several limitations in our study. First, the data group was small; further investigation using a larger data set is needed. Second, various parameters of the immune system can effect the action of radioprotector agents, therefore, it seems these parameters must be evaluated separately.

Conclusion

This study showed selenium and vitamin-E can decrease DNA damage inflicted by x-rays radiation in volunteers' human peripheral blood lymphocytes if it is given before exposure. Selenium and vitamin-E are natural compounds and no side effects found in this study. This study showed that selenium and vitamin-E are effective radio-

protectors because these compounds reduced DNA damage in blood lymphocytes. This study has produced a new data set contribute to the growing body of evidence about radiation protection efficiency of selenium and vitamin-E (separately and synergistically) and confirms that synergistically use these compound can be more effective than separate use of them. The results also suggest that simultaneous ingestion of these radioprotective agents could increase the radiation protection efficiency of each one.

However, before they are approved for clinical use, further experimental studies by using different cytogenetic must be done. Moreover, conjugation of this compound with monoclonal antibody can increase the effect of these compounds in cancer radiotherapy.

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