Original Article

The Radioprotective Effects of Melatonin and Nanoselenium on DNA Double-Strand Breaks in Peripheral Lymphocytes Caused by I-131

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Abstract

Background: One of the treatment modalities for thyroid cancer and hyperthyroidism is radioiodine-131 (I-131) therapy. The use of this therapeutic modality is not completely safe and can lead to oxidative stress, eventually DNA damages. However, these radiation-induced damages can be reduced by antioxidants. This study aimed to investigate the potential radioprotective effects of melatonin and selenium nanoparticles (SeNPs) on DNA double-stranded breaks (DSBs) caused by I-131. **Materials and Methods:** After obtaining informed consent, 6 ml blood was taken from each volunteer. The samples were divided into two general groups of control (without I-131) and with I-131. Each group was also divided into three subgroups, including without antioxidant, melatonin, and SeNPs. The samples of control group were incubated for 2 h after adding the antioxidants. The samples of I-131 group were first incubated for 1 h with the antioxidants and then the samples re-incubated for another 1 h after adding the I-131. Then, the samples were prepared for γH2AX assay. **Results:** The findings showed that after 1 h of incubation with 20 μCi I-131/2 mL, the DSB levels increased by 102.9% in comparison with the control group. In the I-131 group, there were significant reductions of the DSB levels after incubation with melatonin ($P < 0.001$) and SeNPs ($P < 0.001$) in comparison with the without antioxidant subgroup. Furthermore, the DSB levels at the melatonin + I-131 and the SeNPs + I-131 subgroups decreased to 38% and 30%, respectively, compared to the I-131 subgroup. **Conclusion:** According to the obtained findings, it can be concluded that the use of melatonin and SeNPs (as radioprotector agents) can reduce the DSB levels induced by I-131 in peripheral lymphocytes.

Keywords: *γH2AX, double-strand break, melatonin, nanoselenium, nuclear medicine*

Introduction

The potentials of radionuclides, such as I-131, in the treatment of malignant tumors and pain control have been known for many years.[1] I-131 is used to treat hyperthyroidism and thyroid cancer, as it has been applied as an effective treatment for over half a century.[2] Although thyroid cancer is one of the less common malignancies (only 1%–2% of all cancer types), the prevalence of this cancer has grown dramatically throughout the world in recent decades and it is considered to be the most common endocrine cancer.[3-5]

Ionization radiation emitted from I-131 causes irrecoverable damage to important intracellular targets such as DNA, RNA, proteins, and lipids, eventually leading to cell death.[6] Furthermore, it can lead to complications such as neck pain, swelling, and organ dysfunction (including problems with the pulmonary system and gastrointestinal, hematopoietic, and salivary glands).[7-9] At high-dose values, there are more serious adverse effects such as leukemia, pneumonia, pulmonary fibrosis, infection, and severe bleeding.[10,11] During I-131 therapy, serious radiation damage to the DNA of healthy cells can also lead to an increase in radiation-induced secondary cancers.[2,12,13] Double-strand breaks (DSBs) are the most notable type of cell radiation damage which are caused by the inability to properly repair, as this defect in repair process can lead to mutations and cancer.[14] It is noteworthy that the number of these DSBs remains high even after 144 h following I-131 therapy;[15,16] in this regard, γH2AX assay can be used as a sensitive and accurate method for identifying the DSBs caused by radiation.[17]

Radioprotector agents can be used to prevent or reduce DNA DSBs in peripheral lymphocytes induced by

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radiation.[18-22] For instance, it has reported that melatonin can enhance DNA repair induced by ionizing radiation, chemotherapeutic drugs, and some other mutagens which this property of melatonin is directly related to its interaction with enzymes involved in DNA repair.[23-26] In this regard, for example, this radioprotector through direct phosphorylation of Ser15 in p53 inhibits cell proliferation, providing more time for repairing the damaged DNA; hence, the accumulated DNA damage is avoided as well as cell death or probability of genomic instability is reduced.[27] Melatonin (N-acetyl-5-methoxytryptamine) is a small lipophilic molecule that is secreted by the pineal gland, and it is responsible for the regulation of some specific physiological functions in the body.[28,29] The radioprotective effects of melatonin have been studied by some researchers.[30-33] It has various antioxidative, antiapoptotic, and anti-inflammatory properties and can alleviate radiation-induced damages.[34] Melatonin exerts antioxidative properties through two various mechanisms (direct and indirect pathways).[35]

Selenium is a trace element in the human body that its low level is essential. The potent antioxidant and radioprotective effects have been proven for many years.[36,37] Selenium nanoparticles (SeNPs) are less toxic than selenium compounds, and they can neutralize reactive oxygen species such as DPPH and superoxide anion.[38] It is notable that the ability of the nanoparticles depends on their sizes, as small SeNPs have higher free radical scavenging potential.[39]

The radioprotector effects of melatonin have been examined in several studies, but there is no information on its effects on DNA DSBs caused by I-131. There is also little information about the protective effects of SeNPs on DNA damages.[40,41] Therefore, the present study aimed to evaluate the possible radioprotective effects of melatonin and SeNPs on DNA DSBs in peripheral lymphocytes irradiated by I-131.

Materials and Methods

Study design and sampling

This experimental protocol was approved by the Ethics Committee of Kashan University of Medical Sciences, Kashan, Iran (IR. KAUMS. REC.1395.81). Written informed consent forms were signed by blood donors. The inclusion criteria of the study were being healthy, nonsmoker, being aged between 18 and 60 years, and without history of chemotherapy or medical irradiation at least 2 weeks before the sampling period. The individuals were five healthy volunteers (four males and one female) aged 21–48 years (mean: 37 years), with a mean body mass index of 24 kg/m^2 (range: 19–39 kg/m^2). Blood samples were taken from median cubital veins of the participants (6 mL/participant) and collected in tubes containing heparin anticoagulant. Then, each sample was divided into six equal parts and poured into heparin tubes.

Following the incomplete factorial method, the tubes were divided into two major groups: control (without I-131) and irradiated with I-131. Each group was classified into three subgroups: without antioxidant, melatonin, and SeNPs. In other words, the six subgroups were as follows: (1) control, (2) melatonin, (3) SeNPs, (4) I-131, (5) melatonin + I-131, and (6) SeNPs + I-131.

Antioxidants

Melatonin and SeNPs were purchased from Sigma-Aldrich and American Elements Companies. Assuming there is 6 L of blood in the human body (with the mean weight of 75 kg), 0.0167 mg melatonin per mL of blood[22] and 0.025 mg SeNPs per mL of blood[40] were calculated in accordance with existing studies. These values were accurately calculated and added to the subgroups.

Treatment and irradiation

The samples of control group, after adding the melatonin and the SeNPs, were incubated for 2 h at 37°C. The samples of with I-131 group were also incubated for 1 h with the antioxidants at 37°C. Then, 20 μCi I-131 (provided by the Nuclear Medicine Center of Kashan Shahid Beheshti Hospital, Iran) and normal saline (NaCl), in a total volume of 1 mL, were added to the samples and the final volume of each vial was 2 ml. After incubation, the samples containing the I-131 were centrifuged at 2000 g for 15 min. Lymphocyte sample preparation, γH2AX immunostaining, and quantification were essentially done as described previously.[15,16] Peripheral blood lymphocytes were isolated by ficoll-hypaque using the protocol suggested by the manufacturer. The blood samples were diluted 1:1 with phosphate-buffered saline (PBS) and layered onto ficoll-hypaque solution with the ratio of blood and PBS: ficoll-hypaque maintained at 2:1. The blood samples were centrifuged at 2000 rpm for 15 min at 4°C (a temperature with minimal reduction of foci). The lymphocyte layer was removed, washed twice with PBS, and centrifuged at 2000 rpm for 10 min at 4°C. Afterward, they were fixed in 4% paraformaldehyde for 15 min at room temperature and were then washed twice with PBS for 5 min each. After washing, approximately 20 μl of the solution was duplicated on the slide followed by permeabilization in cold acetone for 10 min. The cells were washed 3 times with PBS as well as for 15 min in blocking solution (PBS with 5% bovine serum albumin [BSA] and 0.2% triton x-100) at room temperature. The cells were stained overnight by a specific γh2ax antibody (Millipore, Germany, clone jbw301) (dilution 1:500 in PBS containing 1% BSA and 0.05% x-triton) at room temperature and were washed afterward with PBS for 10 min. The slides were incubated with the secondary antibody (Alexa Fluor 488) (dilution 1:500) for 60 min in a dark chamber at the room temperature. Subsequently, they were washed three times with PBS for 15 min and mounted with propidium iodide (dilution 1:50) (Invitrogen Co.).

Enumeration of γH2AX foci was accomplished using a fluorescent microscope (Ceti, UK) equipped with a ×100 magnification objective by two blind observers. The samples were counted continuously for at least 100 cells. Granulocytes and monocytes were omitted by morphological criteria, and the average number of DSB/cell was calculated.

Statistical analysis

After analyzing the normality of the data by the Kolmogorov–Smirnov test, the mean and standard deviation (SD) of DSBs for each subgroup were calculated. Moreover, an increased level of DSBs was obtained using Eq. (1):

$$\frac{(\text{with I-131}) - (\text{without I-131})}{\text{without I-131}} \times 100$$

The decreased level of DSBs was also obtained using Eq. (2):

$$\frac{(\text{with I-131}) - (\text{with I-131 + melatonin or SeNP})}{\text{with I-131}} \times 100$$

Statistical differences between the subgroups were assessed using independent *t*-tests and one-way analysis of variance (ANOVA). Furthermore, to assess multivariate effects, a factorial design in accordance with a generalized linear model (GLM) was applied. Finally, $P < 0.05$ was considered statistically significant.

Results

The findings showed that the mean ± SD of DSBs/cell at the control subgroups of without antioxidant, melatonin, and SeNPs was 0.169 ± 0.031, 0.163 ± 0.029, and 0.160 ± 0.020, respectively [Figure 1]. There was no significant difference between the samples of the nonirradiated subgroups ($P = 0.66$) [Table 1].

The levels of γH2AX foci induced by I-131 were enumerated 60 min after the incubation. The range of DSBs/cell at the irradiated subgroups of without antioxidant, melatonin, and SeNPs was 0.310–0.378 (mean ± SD: 0.343 ± 0.023), 0.182–0.245 (mean ± SD: 0.211 ± 0.024), and 0.227–0.275 (mean ± SD: 0.242 ± 0.027), respectively [Figure 1]. The results of one-way ANOVA revealed significantly higher focus levels induced by I-131 compared with the control group ($P < 0.001$). Compared to the I-131 + without antioxidant subgroup, the focus levels after incubation with melatonin and SeNPs reduced to 38% ($P < 0.001$) and 30% ($P < 0.001$), respectively. However, there was no significant difference between the melatonin + I-131 and the SeNPs + I-131 subgroups ($P = 0.95$).

Furthermore, a GLM was employed for the multivariate analysis of effects of melatonin and SeNPs in the absence or presence of I-131. The findings of GLM revealed the effect of various groups on the levels of DSBs in the presence or absence of I-131. Moreover, the interactive effect of antioxidants on the DSB levels was assessed in accordance with the presence or absence of I-131 ($P < 0.001$).

Fluorescence microscopy images of γH2AX foci in lymphocytes irradiated with I-131 are shown in Figure 2.

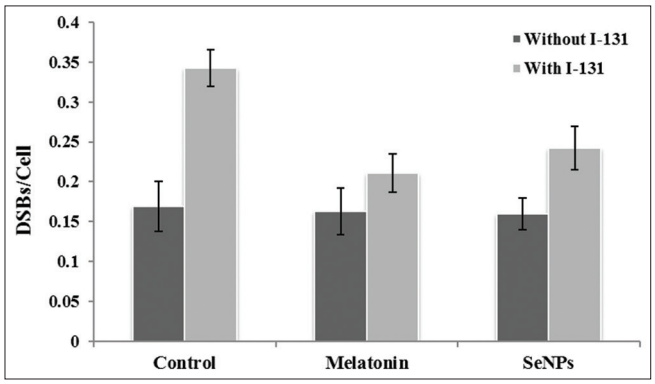

Figure 1: The DNA double-strand breaks per cell in peripheral lymphocytes for various subgroups of control, melatonin, selenium nanoparticles, I-131, melatonin + I-131, and selenium nanoparticles + I-131. The data are shown as the mean ± standard deviation

Discussion

I-131 emits two types of radiation: beta-radiation for treatment and gamma-radiation for diagnoses, as its beta component (with energy 606 Kev) is applied to treat hyperthyroidism and thyroid cancer.[42] In the present study, the potential effects of melatonin and SeNPs (as radioprotector agents) on genetic damages induced by I-131 were investigated.

In the past few decades, the use of radiological and nuclear medicine procedures has increased dramatically.[43] Ionizing radiation not only destroys malignant cells but also damages healthy tissues. The most important damage caused by radiation is DNA DSBs which can lead to genetic abnormalities and cell death due to defects in

Table 1: The mean±standard deviation of double-strand breaks in the different groups

Group	Without I-131	With I-131	*P*
Control	0.169±0.031	0.343±0.023	<0.001
Melatonin	0.163±0.029	0.211±0.024	<0.001
Selenium nanoparticles	0.160±0.020	0.242±0.027	<0.001
P	0.66	<0.001	

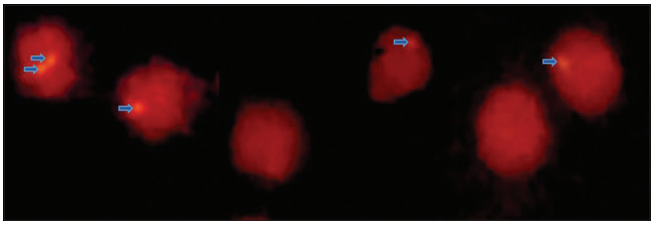

Figure 2: Microscopic image (×1000) of γH2AX foci after *in vitro* irradiation with I-31. Each of the tiny dots represents one DNA double-strand break (arrow)

repair process.[15] A biomarker for DSB formation is the phosphorylated form of Ser139 of the minor histone H2 variant H2AX (γH2AX).[44] There is a direct relationship between the number of foci and the absorbed dose value in computed tomography scan, radiation therapy, or systemic radionuclide therapy, even for absorbed dose values to the blood below 20 mGy.[15,16,45,46]

According to a previous study, the radiation dose received by a patient following I-131 is about 40 mGy, as this dose value is resulted from 20 μCi of I-131.[47] Furthermore, the greatest damage occurs in the first 2 h after receiving I-131.[15,48] Therefore, 20 μCi I-131/2 ml (1 mL blood + 1 mL normal saline containing I-131) was used in the current study. The number of DSBs/cell recorded after 2 h was 0.343 ± 0.023; this value was slightly different from that of reported by Lassmann *et al.* (median: 0.227 DSB/cell).[15] This difference may be due to the study type (*in vivo* vs. *in vitro*).

Our findings showed that melatonin can reduce the I-131-induced DNA DSBs by 38%. The radioprotective effect of melatonin has been proven in previous studies. In a study by Esmaely *et al*.,[22] the effect of melatonin on levels of DSBs induced by X-ray in peripheral lymphocyte using H2AX immunofluorescence microscopy was investigated. They reported that 100 mg melatonin reduced the DSB levels up to 39% and 33% for 10 mGy and 100 mGy following 1st h pretreatment. The difference between the findings of our study and Esmaely *et al*. may be due to (1) study type (*in vivo* and *in vitro* vs. *in vivo*), (2) radiation dose value used (10 and 100 mGy vs. 40 mGy), and (3) radiation source used (X-rays generated from an external source with 50 kV vs. beta- and gamma-rays emitted from I-131). In another study, Rostami *et al*.[44] evaluated the radioprotective effects of Vitamin C and melatonin against genotoxicity induced by 6 MV X-rays in human lymphocytes. Their results revealed that total micronucleus values induced by radiation reduced to 55%, 65%, and 43% of initial values following 1 h after oral ingestion of 300 mg melatonin, 300 mg Vitamin C, and 300 mg melatonin + 300 mg Vitamin C, respectively. The difference between their results and our findings may be due to (1) study type (*in vivo* vs. *in vitro*), (2) radiation dose value used (20 cGy vs. 40 mGy), and (3) radiation source used (X-rays generated from an external source with 6 MeV *vs.* beta- and gamma-rays emitted from I-131).

Other results of the current study demonstrated that the use of SeNPs reduces the I-131-induced DNA DSBs by 30%. It is notable that the radioprotective effect of SeNPs on damages caused by radiation (I-131) was first evaluated in this study. There are limited studies which have examined the effectiveness of SeNPs against damages caused by oxidative stress, especially in human blood samples. In a study by Zhang *et al*.,[40] protective effects of SeNPs on nickel sulfate-induced testicular damage and apoptosis in rat testes were evaluated. The histopathological results demonstrated that the SeNPs improved damages of testicular tissue induced by nickel sulfate. Furthermore, SeNPs significantly alleviated nickel sulfate-induced apoptosis in rat testes. Furthermore, these nanoparticles significantly upregulated Bcl-2 and downregulated cytochrome c, Bak, caspase-3, and caspase-9 expression levels. In another study, Rezvanfar *et al*.[41] assessed the protective effect of SeNPs on reproductive toxicity induced by cisplatin chemotherapy drug in Wistar rats. They represented that co-administration of SeNPs significantly improved the serum testosterone, sperm quality, and spermatogenesis and decreased cisplatin-induced spermatic DNA injury and free radical toxic stress.

The results presented in the current study was obtained *in vitro*, therefore, *in vivo* and clinical trials are needed in the future to confirm the effectiveness of melatonin and SeNPs against damages induced by I-131. Furthermore, we examined the radioprotective effects of melatonin and SeNPs separately; hence, it is suggested to evaluate the potential synergic effect of melatonin and SeNPs on damages induced by I-131. Furthermore, assessment of the radioprotective effects of these agents during the presence of I-131 in the body is suggested as a future study.

Conclusion

The findings of the present study showed that the use of melatonin and SeNPs in irradiated blood samples with 40 mGy of I-131 can reduce the levels of DSBs in peripheral lymphocytes. In addition, it was found that the radioprotective effect of melatonin against I-131-induced damages is more than SeNPs.

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Conflicts of interest

There are no conflicts of interest.

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