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

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 µg 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. I-131 is used to treat hyperthyroidism and thyroid cancer, as it has been applied as an effective treatment for over half a century. 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.

Ionization radiation emitted from I-131 causes irreversible damage to important intracellular targets such as DNA, RNA, proteins, and lipids, eventually leading to cell death. 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). At high-dose values, there are more serious adverse effects such as leukemia, pneumonia, pulmonary fibrosis, infection, and severe bleeding. During I-131 therapy, serious radiation damage to the DNA of healthy cells can also lead to an increase in radiation-induced secondary cancers. 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. It is noteworthy that the number of these DSBs remains high even after 144 h following I-131 therapy in this regard, γH2AX assay can be used as a sensitive and accurate method for identifying the DSBs caused by radiation.

Radioprotector agents can be used to prevent or reduce DNA DSBs in peripheral lymphocytes induced by...
It is notable that the range: 19–39 kg/m². The peripheral blood lymphocytes were isolated by ficoll-hypaque using the protocol suggested previously. 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. 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 then were 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 x-100) 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.).
Jafarpour, et al.: Effects of melatonin and nanoselenium on DNA DSBs

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.

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. 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. 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

| 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 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.

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).
A biomarker for DSB formation is the phosphorylated form of Ser139 of the minor histone H2 variant H2AX (γH2AX). There is a direct relationship between the number of foci and the absorbed dose value in computed tomography scan, radiation therapy, or systemic radiotherapy damage, even for absorbed dose values to the blood below 20 mGy. 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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Jafarpour, et al.: Effects of melatonin and nanoselenium on DNA DSBs

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