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Radiosensitizing effects of melatonin on radiation induced chromosomal aberration in G2-lymphocytes of breast cancer patients

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ABSTRACT

Background: Radiotherapy is regarded as a standard treatment modality in breast cancer (BC). Radiation causes cellular damage both in cancer and normal cells by inducing DNA-damage and chromosomal aberrations (CA). Different agents were used for ameliorating effects of radiation, mainly antioxidants such as melatonin. Melatonin shows oncostatic properties on human BC. The aim of this study was to evaluate the modulating effect of melatonin on radiation induced CA in cells irradiated at G2 phase of the cell cycle. ***Materials and methods:*** G2 assay was applied on whole peripheral blood lymphocytes received from 10 BC patients and 5 normal controls. Blood culture was initiated in complete culture medium. Four h prior to harvesting, cells were irradiated with 1Gy gamma rays. Pretreatment of samples with melatonin was done 3 h before irradiation. After metaphase preparation and slide making, slides were stained in Giemsa. Hundred well spread metaphases were scored for the presence of chromatid type aberrations with a microscope at a magnification of × 1000. ***Result:*** Results indicated a high and significant frequency of CA both in lymphocytes of normal and breast cancer patient after irradiation; however, the frequency was much more in lymphocytes from BC patients. Pretreatment of samples with melatonin led to a considerable increase in the frequency of aberrations especially in lymphocytes of BC patient. ***Conclusion:*** Results showed that despite having antioxidant property, melatonin led to enhanced frequency of radiation induced CA in lymphocytes of BC patients.

Keywords: *Melatonin, breast cancer, chromosomal aberration, radiation therapy, G2 assay.*

INTRODUCTION

Breast cancer is one of the most common neoplasia occurring in women mainly in developed countries that ranks as the fifth cause of death from all cancer [(1)]. Breast cancer risk factors includes gender and age, family history, genetics, hormone therapy, exposure to ionizing radiation, dietary habits, obesity and alcohol and tobacco consumption as well as circadian disruption [(2)]. Radiotherapy is one of the most common treatments for breast cancer. About 80% of patients with BC receive RT.

Radiation causes DNA damage directly or indirectly through ROS formation. The creation of ROS generates not only DNA strand breakages but also might act as a signaling event leading to the release of cytokines or epigenetic changes or trigger DNA repair machine [(3)]. Ionizing radiation can cause cell cycle arrest which allows time for DNA repair and prevents the progression of damaged cells from the G2 phase into mitosis [(4)]. Oxidative stress as ROS is involved in the etiology of many diseases

including cancer [5]. Unfortunately radio sensitivity of normal tissues contiguous to the tumor limits therapeutic benefit. Some of the patients show different degrees of mild to acute reactions.

Antioxidants are compounds, which combat the free radicals produced during radiation therapy. Antioxidants protect cells against development of cancer [5]. Therefore, it seems logical to use antioxidant adjuvants to reduce radiotherapy toxicity [6].

Melatonin has been shown to have radio protective and anticancer effects. Antioxidant properties of melatonin based on free radical scavenging activity have been established in various experimental models [7]. Melatonin (N- acetyl -5 methoxy tryptamin) is an indoleamin secreted mainly by the pineal gland during the dark hours at night. Numerous studies have been performed to evaluate the oncostatic properties of melatonin against different neoplasias [8]. Experimental studies carried out in rodents have shown that melatonin prevents the promotion and growth of mammary tumors [9-12]. Also melatonin as an adjuvant therapy, conserves against the side effects of chemotherapeutic drugs [13]. Disruption of nocturnal melatonin secretion is a risk factor for breast cancer [14]. Cohen *et al.* (1978) proposed that decrease in melatonin levels might promote the development of breast cancer in human. Melatonin have properties of antioxidant reduces the side effects of radiotherapy [15] by scavenging ROS and reactive nitrogen species [16]. The role of melatonin is to excite the expression of anti-oxidative enzyme [17] and deducting the expression of pro-oxidative enzymes [18].

Melatonin acts in different pathways involved in cancer treatment including, cell cycle regulation, differentiation, telomerase inhibition, apoptosis, metastasis, prevention of circadian disruption, and other related antioxidant properties [15]. Although, other reports have also shown radiosentisizing effect of melatonin [19].

Melatonin pertains to the antioxidant group of radioprotectors [20]. Melatonin may postpone the inhibition of the repair enzymes, hence allowing the repair of induced damage and the use of higher doses of radiation may supply better therapeutic value [20]. Experimental research established the protective effect of melatonin against the genetic damage in blood, bone marrow and mortal effect of whole body radiation in mice [21].

Because of the controversies over the effect of melatonin, the aim of this research was to examine the effect of melatonin on G_2 lymphocytes of breast cancer patients and normal healthy individuals after irradiation to gamma rays.

MATERIALS AND METHODS

Study subjects

Whole blood sample was collected from 10 breast cancer patients with the mean age of 53.3±10.3 (age range 37-78) and 5 healthy normal individuals with mean age of 39.6 ± 11.6 (aged 28-63) in heparinized tubes. The research was authorized by the institutional ethical committee. All donors gave their informed written consent and completed a written questionnaire to give information related to their personal life style including dietary habits, medical history and exposure to chemical and physical agents. Hence, to limit confounding factors, all samples were scrutinized to exclude former radiation exposure, antibiotic therapy and virus infection at least one month prior to sampling. Patients with breast cancer were not under chemo or radiotherapy treatment and all were diagnosed as new cases.

Blood culture, melatonin treatment and irradiation

Each sample from patient or healthy normal individual was divided into four parts: one part left without any treatment (Control, C), the second part was treated with melatonin alone (M), third part was irradiated alone (R), and the forth one received melatonin 3h before radiation (R+M). To each culture 0.4 ml blood was added to 3 ml RPMI-1640 Medium, supplemented with 15% fetal bovine serum, 1% L-glutamine and antibiotics (penicillin 100 iu/ml and streptomycin 100 µg/ml) (all reagents from

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Gibco BRL).

The lymphocytes were stimulated to divide with 1% phytohemagglutinin (PHA)(Sigma). Whole blood cultures were incubated at 37 ˚C, sixty-six hours later, melatonin (Chemidarou pharmaceutical co, Tehran, Iran) was added at a final concentration of 25 µg/ml to some samples. After 3 hours culture vessels were exposed to 1 Gy gamma rays generated from a ^{60}Co source (ACEL, Canada) at a dose rate of 85 cGy / min. Slides were dried and stained in 4% Giemsa for 10 min. Chromatid breaks were scored and analyzed in well spread metaphase cells under ×100 oil immersion light microscope. 100 cells were scored per sample. Figure 1 shows sample metaphase spreads with and without chromatid breaks.

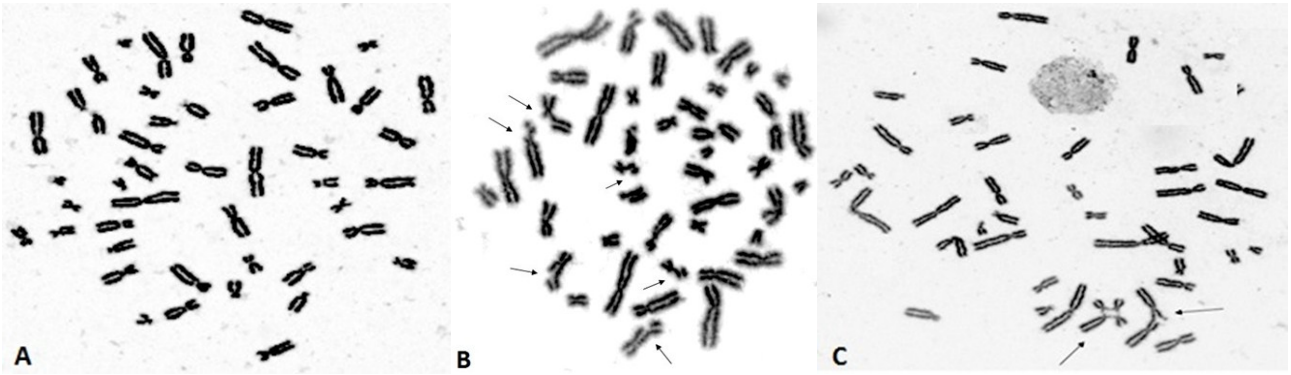


Figure 1. A typical photomicrograph showing normal metaphase **(A);** metaphase with chromatid breaks (arrows) **(B)** and metaphase with rare exchange aberrations (triradial and quadriaradial, arrows). Magnification×1000.

Statistical analysis

SPSS (version 18, Chicago, IL, USA) was used to do statistical analysis on data obtained. Non- parametric Mann-Whitney U-test one way analysis of variance (ANOVA) was used to test the significant difference between studied groups. P-value less than 0.05 was regarded as significant level.

RESULTS

The data obtained from normal control and breast cancer patients before and after 1Gy gamma irradiation with and without melatonin is summarized in table 1 and shown in figures 2 and 3.

Results of patients

As seen in figure 3, the effect of melatonin alone was more pronounced compared to background frequency of chromatid breaks in lymphocytes of patients statistically significant ($p<0.05$). The frequency of radiation induced chromatid breaks was significantly higher than control ($p<0.01$). Moreover, pretreatment of lymphocytes with melatonin led to an increased in frequency of radiation induced aberration ($p< 0.01$).

Table 1. Mean frequency of chromatid breaks scored in blood samples obtained from control individuals and breast cancer patients. SD indicates standard deviation of mean values.

Subjects	No. of samples	Mean ±SD age (Range)	No. of cells analyzed	Mean ±SD background breaks	Mean ± SD break induced Melatonin alone	Mean ± SD gamma rays induced breaks	Mean ± SD gamma rays induced breaks in presence of melatonin
Control	5	37.4± 11 (23-66)	500	1.4 ± 1.02	2.14 ± 1.06	14.0 ± 9.14	15.2 ± 6.05
Breast cancer patient	10	46± 13.9 (25-76)	1000	5.6 ± 3.4	15.1 ± 22.23	72.1 ± 27.85	119 ± 46.67

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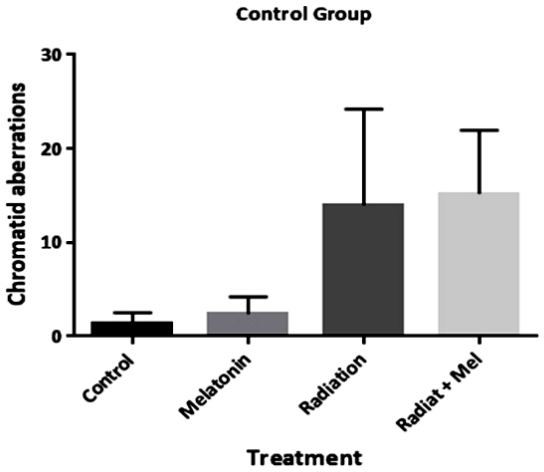


Figure 2. Frequency of chromatid aberrations observed in lymphocytes of normal controls. Error bars Indicate standard deviation of mean values.

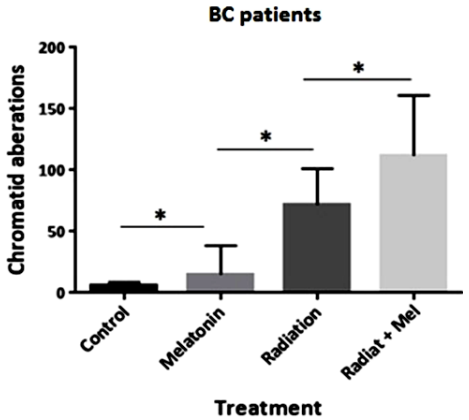


Figure 3. Frequency of chromatid aberrations observed in lymphocytes of BC patients. Error bars Indicate standard deviation of mean values.

DISCUSSION

The G_2 chromosomal aberration assay is a well-accepted method to study interaction of radiation and chemical agents. Result of the study presented in table 1 and figures 2 and 3 indicate that lymphocytes of breast cancer patients received melatonin 3 h before irradiation show an elevated frequency of chromatid aberration compared to cells received radiation alone; suggesting that the addition of melatonin promotes chromatid breaks and enhances the effects of radiation.

Useful properties of melatonin in the prevention and treatment of breast cancer were reviewed in Mediavilla *et al.* [22]. These properties include antioxidant effects [23], regulation of the estrogen receptor expression [24], regulation of the enzymes involved in the synthesis of estrogens [25-27], modulation of cell cycle and apoptosis [28] as well as inhibition of telomerase activity [29]. Moreover it was shown that melatonin stimulates cell differentiation [30], prevents angiogenesis [31], inhibits metastasis [32], prevents circadian disruption [33], and reduces epigenetic effects [34, 35]. Melatonin by its antioxidant properties reduces the side effects of radiotherapy [15] by scavenging ROS and reactive nitrogen species [16]. Moreover, melatonin triggers the expression of antioxidant enzyme [17] and reduces the expression of pro-oxidative enzymes [18]. Although melatonin was previously considered as an antioxidant agent, particularly as scavenger of ROS, our results demonstrate that melatonin could act as a radiosensitizer. Similar results with slight variations were reported previously. Alonso –Gonzalez [19] reported melatonin pretreatment before radiation sensitizes breast cancer cells to the ionizing effects of radiation by downregulation proteins (RAD51, DNA-PKcs) involved in double –strand DNA break repair. It has been shown that melatonin can also reduce the effectiveness of DNA repair and increase the rate of DNA damage promoted by irinotecan, a camptothecin analog used in clinic for treatment of different malignancies, in human non-small-cell lung cancer and human colorectal adenocarcinoma cell lines [36]. As well as, melatonin by decreasing the ratio of cells in the S and G2 phases decrease the probability to repair the DNA damage by homologous recombination, which occur in these phases of the cell cycle in breast cancer [37]. However, the accurate roles of p53 in repair of DSBs stand controversial, there are evidences for a straight role of p53 in homologues recombination as well as in nonhomologous DNA end joining [37, 38]. Melatonin augments the expression of P21WAF1 and p53 [39], two regulatory proteins of the cell cycle, in BC cell, and this mode of action of melatonin could be related to its modulatory effect on DNA repair.

In addition to the antioxidant mechanisms of melatonin and protection of DNA, our results show that melatonin influence on the

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radio-sensitivity of lymphocytes of breast cancer patients. Free radical scavenging activity of melatonin has been reported previously [40].

In conclusion results of the present study indicate that despite antioxidant property of melatonin, treatment of cells with melatonin before irradiation led to an increased clastogenic effect in lymphocytes of BC patients. The mechanism by which melatonin enhanced radiation effect is not fully understood but may be due to alterations in genes involved in DNA repair process leading to increased chromatid aberration.

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Conflicts of interest*: *Declared none.

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