Investigation of Genotoxic Effect of Taxol Plus Radiation on Mice Bone Marrow Cells


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In this study, we investigated the genotoxic effect of taxol, radiation, or taxol plus radiation on highly proliferative normal tissue-bone marrow cells of Swiss albino mice. Swiss-albino mice, 3–4 months old, were used in this study. Taxol was administered bolus intravenously through the tail vein. Radiation was given by using a linear accelerator. There were four treatment categories, which had a total of 34 groups. Each group consisted of five animals. The first was the control category that had one group (n=5). The second treatment category was taxol alone, which had three groups as per taxol dose alone (n=15). The third treatment category was radiation alone, which had three groups as per the radiation dose (n=15). The fourth treatment category was taxol plus radiation, which had 27 groups as per combined radiation dose plus taxol dose concentration and as per pre-treatment timing sequence of taxol before radiation (n=135). Mice were sacrificed 24 h after taxol or radiation or combined administration using ether anesthesia. The cells were then dropped on two labeled slides, flamed, air dried, and stained in 7% Giemsa; 20–30 well-spread mitotic metaphases were analyzed for each animal; the cells with chromosome breaks, acentric fragments, and rearrangements were evaluated on ×1,000 magnification with light microscope (Zeiss axiplan). The mitotic index was determined by counting the number of mitotic cells among 1,000 cells per animal. Differences between groups were evaluated with Student’s t-test statistically. Taxol caused a dose-dependent increase in chromosomal aberrations ($P=0.027$). Similarly, radiation caused a dose-dependent increase in chromosomal aberrations ($P=0.003$) and decreased mitotic index.
In combination, there were a small enhancements at the 40 mg/kg taxol dose level and at 0.25 and 0.5 Gy radiation doses in the 48 h group. However, an increase in chromosomal aberrations was observed after 48 hours of taxol exposure when compared 12 or 24 h of taxol exposure ($P=0.001$ and $P=0.019$). These findings suggest that taxol at the high doses with low dose radiation caused radiosensitizing effect in bone marrow cells. Forty-eight-hour pre-treatment of taxol exposure followed by radiation caused significant induction of chromosomal aberrations and a reduction of mitotic index when compared to other taxol timing sequence. *Teratogenesis Carcinog. Mutagen.* 22:1–11, 2002. © 2002 Wiley-Liss, Inc.

Key words: taxol; radiation; mitotic index; chromosomal aberrations; bone marrow cell

INTRODUCTION

Paclitaxel (Taxol) is a semi-synthetic product derived from the bark of the yew tree *Taxus brevifolia*. Taxol blocks the cell cycle in G2-M phase by preventing polymerization of microtubules. It causes formation of abnormal microtubules and aggregation of microtubules [1–4]. Antitumoral effects of Taxol have been shown in several types of cancers including ovarian, breast, lung, and head and neck cancers [5–8]. It has also been demonstrated that taxol is a potent radiosensitizer in human cancer cell lines [9–17].

The role of the combined effect of taxol and radiation is not clearly understood. It is well known that cells in late G2 and M phases are the most sensitive phases for radiation damage [18–20]. It has been speculated that taxol might sensitize cells to radiation damage by blocking them in G2 and M phases [14,21,22]. In addition to its effect on cell cycle, it has also been shown that Taxol induces apoptosis in human tumor cell lines. Based on this observation, it has been suggested that apoptosis might be an endpoint response to the effects of taxol [14,15,21].

Although all phases of the cell cycle are affected by stabilization of microtubules, the largest effect is seen in mitotically active cells [8,23]. Cells that are affected by cell cycle block or prolonged of cytokinesis may undergo apoptosis or necrosis or can complete mitosis by release of cell-cycle block [21,24,25].

Since the most prominent effect of taxol is on the mitotic phase, tumor cells and rapidly proliferating normal tissues, such as bone marrow and gastrointestinal mucosa, are the most affected [23,25]. Although numerous papers have been published that show increased apoptotic effect of combined taxol and radiation exposure in animal tumors and in several cancer cell lines, there are few studies that have investigated the effects on normal tissue.

Thus, in this study, we investigated exclusively the effects of taxol plus radiation on highly proliferative normal bone marrow cells. Here, we demonstrate that taxol does not confer a radiosensitizing effect on the normal proliferative tissue and this observation may be exploited in the treatment tumors by sparing normal tissue.

MATERIAL AND METHODS

Animals

One hundred seventy in-bred Swiss-albino mice that were obtained from the Test Animals Brooding Center of Uludag University were used for this experiment.
Animals were fed with standard feed and water under controlled sterile hygienic condition. They were 3–4 months old and weighed 20–25 grams. The male/female ratio was equal in all treatment categories. The light and dark cycle was automatically regulated at 12 h. There were four treatment categories, which had a total of 34 groups. Each group consisted of five animals.

**Treatment Categories**

The first category was the control category, which had one group (total n=5). The second treatment category was taxol alone, which had three groups as per taxol dose alone (total n=15). The third treatment category was radiation alone, which had three groups as per the radiation dose (total n=15). The fourth treatment category was taxol plus radiation, which had 27 groups as per combined radiation dose plus taxol dose concentration and as per pre-treatment timing sequence of taxol before radiation (total n=135).

**Taxol and Irradiation Treatment**

Taxol was administered intravenously through the tail vein. Whole body irradiation was given with a special animal-fixing device using a linear accelerator (6 MV photon) at a dose-rate of 300 cGy/min. Dose was calculated manually as $D_{\text{max}}$ dose. The control treatment category did not receive any treatment. In the taxol treatment alone category, each of three groups received 10, 20, and 40 mg/kg, respectively. In the radiation treatment alone category, each of the three groups received 0.25, 0.5, and 1 Gy, respectively. In the taxol plus radiation treatment category, the 27 groups divided as follows: Taxol was administered taxol intravenously at doses of 10 or 20 or 40 mg/kg initially. They were then irradiated at dose 0.25 or 0.5 or 1 Gy after 12 or 24 or 48 h taxol pretreatment.

**Analysis of Mitotic Index and Chromosomal Aberrations**

Mice were sacrificed 24 h after treatment administration by using ether anesthesia. Both femurs of each mouse were dissected and the bone marrow cells were flushed gently with 0.075 KCl solution into the centrifuge tube. The cells were treated for 20 min with hypotonic solution. Following incubation, cells were centrifuged. The pellet was resuspended in cold fixative and tubes were refrigerated for 20 min. Cells were washed in fresh three times before preparing the slides. To prepare slides, the final cell pellet was suspended and a few drops of fresh fixative were added to each tube. The cells were then dropped on two labeled slides, flamed, air dried, and stained in 7% Giemsa (24); 20–30 well-spread mitotic metaphases were analyzed for each animal and the cell with chromosome breaks, acentric fragments, and rearrangements and other types of aberrations were evaluated blindly on $\times 1,000$ magnification with a light microscope (Zeiss axioplan). The mitotic index was determined by counting the number of mitotic cells among 1,000 cells per animal. The mean percentages and standard deviations were calculated. Differences between groups were evaluated statistically with Student’s $t$-test.

Radiation enhancement ratio (RER) for taxol was calculated using the formula defined by Chendil et al. [30]: $\text{TER} = \frac{\text{Aberrant cell ratio of radiation plus taxol}}{\text{Aberrant cell ratio of Radiation alone}}$. Taxol enhancement ratio (RER) for radiation
was calculated by using the formula: \( \text{RER} = \frac{\text{Aberrant cell ratio of Taxol plus radiation}}{\text{Aberrant cell ratio of Taxol alone}} \). An RER or TER of > 1 indicated radiosensitization or chemosensitization to taxol or radiation, respectively.

RESULTS
Low Chromosomal Aberration Ratio and High Mitotic Index in Bone Marrow Cells of Control Mice Group

In the control group, five mice were used to obtain the baseline frequency of mitotic and apoptotic index. The results of this control group showed a mitotic index mean of 6.0 and an damaged cell ratio mean of 0.4. These results suggest a high frequency of mitotic index and low frequency of damaged cell ratio in the untreated control group.

Taxol Caused an Increased Percentage of Damaged Cells in Bone Marrow Cells

To understand the effect of taxol alone on normal proliferative cells, we exposed mice to 10 or 20 or 40 mg/kg taxol doses and analyzed for mitotic index and percentage of chromosomal aberration in the bone marrow cells. Interestingly, taxol caused significant increase in the frequency of chromosomal aberrations when compared to control group as a dose dependent manner \((P<0.000; \text{Fig. 1})\). These results indicate that taxol exerts a damaged effect on normal proliferative bone marrow cells and can cause mitotic arrest.

![Fig. 1. Chromosomal aberration ratio, damaged cell ratio, and mitotic index of taxol exposure in bone marrow cells. The data shown here is a mean value of the chromosomal aberration ratio, damaged cell ratio, or mitotic index scored from five mice. The error bars represent standard deviation.](image-url)
Ionizing Radiation Caused Dose Dependent Increase in the Percentage of Damaged Cells in Normal Bone Marrow Cells

To understand the effect of ionizing radiation alone on normal proliferative cells, we exposed mice to total body irradiation at 0.25 or 0.5 or 1 Gy doses and analyzed for mitotic index and ratio of damages in the bone marrow cells. Radiation caused a significant dose-dependent increase in the damaged cell ratio when compared to the control group ($P<0.000$; Fig. 2). With 0.25, 0.5, and 1 Gy doses of radiation, a significant dose-dependent decrease in mitotic index was observed when compared to the control group ($P<0.000$; Fig. 2). Together, these results suggest that ionizing radiation can be a potent inducer of chromosomal aberrations.

Low Frequency of Chromosomal Aberrations in Bone Marrow Cells Exposed to Taxol Plus Radiation

To understand the effect of combined treatment, we exposed mice to different doses of taxol followed by different doses of radiation exposure after 12, 24, and 48 h of taxol pretreatment. The frequency of chromosomal aberrations in this combined treatment groups was significantly lower at all time points when compared to either radiation alone ($P<0.000$, Figs. 3, 4). There are small enhancements at 0.25 and 0.5 Gy radiation doses and 40 mg/kg taxol dose in the 48 h group (1.20 for 40 mg/kg + 0.25 Gy/48 hours and 1.83 for 40 mg/kg + 0.5 Gy/48 h). None of other treatments, at all time points, fail to show taxol enhancement ratio of greater than 1 (Table I).

![Graph](image-url)  
**Fig. 2.** Chromosomal aberration ratio, damaged cell ratio and mitotic index of radiation exposure bone marrow cells. The data shown here is a mean value of the chromosomal aberration ratio, damaged cell ratio, or mitotic index scored from five mice. The error bars represent standard deviation.
Meanwhile radiation enhanced the genotoxic effect of taxol, especially in the 48 h groups (range 1.03–2.77). Within the treatment groups and the times of radiation exposure, increased damaged cell ratio was pronounced 48 h after taxol pretreatment ($P<0.000$).

No significant change in mitotic index was observed in all the combined treatment groups (Fig. 5). When compared to the taxol alone group or the radiation alone group, the frequency of the mitotic index was significantly lower in all the combined treatment groups (taxol alone vs. taxol plus radiation, $P=0.000$; radiation alone vs. taxol plus radiation, $P<0.000$). However, within the combined treatment groups, 48 h showed a significant decrease in mitotic index when compared to the 12 and 24 h groups ($P=0.005$ and $P=0.005$, Fig. 5).

Together, these data demonstrate that radiation plus taxol may induce chromosomal aberrations at high taxol and low radiation dose levels and may not alter the mitotic index.

**DISCUSSION**

In addition to currently used modalities, such as surgery, radiation therapy, and chemotherapy, various combinations have recently been under investigation in order
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Fig. 4. Damaged cell ratios caused by taxol plus radiation in bone marrow cells. The data shown here were obtained from 27 groups of five animals each based on combination of radiation doses plus taxol doses and taxol pretreatment timing sequence. The error bars represent standard deviation.

TABLE I. Enhancement Ratios of Taxol and Radiation

<table>
<thead>
<tr>
<th>Taxol plus radiation</th>
<th>Taxol enhancement</th>
<th>Radiation enhancement</th>
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<tbody>
<tr>
<td></td>
<td>12 h</td>
<td>24h</td>
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<tr>
<td>10mg/kg + 0.25 Gy</td>
<td>0.21</td>
<td>0.35</td>
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<tr>
<td>10mg/kg + 0.5 Gy</td>
<td>0.49</td>
<td>0.27</td>
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<tr>
<td>10mg/kg + 1 Gy</td>
<td>0.28</td>
<td>0.05</td>
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<td>20mg/kg + 0.25 Gy</td>
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<td>20mg/kg + 0.5 Gy</td>
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<tr>
<td>40mg/kg + 1 Gy</td>
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to increase the cure rates in cancer patients. Numerous basic and clinical trials have been performed that investigate optimal chemotherapeutic agents, doses, and timing to achieve maximum radiosensitization. Thus, taxol and radiation in combination has been a focus of interest recently [26].
It is well known that radiation is the most effective when cells are in G2 and M phases [18–20]. Taxol is well known to block the cell cycle in G2/M phases. Thus, a combination of these two agents results in enhanced radiation sensitization effect [14,21,22]. Based on this, there are several reports in the literature which have demonstrated that both taxol and radiation may induce apoptosis and act synergistically [9–12,14,15,17].

In contrast, there are very few reports on the radiosensitization effect of taxol on normal tissues. Few studies have shown that taxol did not exert radiosensitizing effects on normal tissues. Interestingly, a protective effect by blocking normal tissue response was reported [27]. Although it is speculated that cells blocked by taxol disappear by some apoptotic mechanisms, the outcome of these cells, such as rates of cells that undergo apoptosis and rates of cells that complete the mitotic cycle, is not clear.

Since taxol primarily affects the microtubule component of the mitotic appara-
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tus, taxol will influence the radiation response of highly proliferative and acutely responding normal tissues. The effects of taxol has been tested mostly on two acutely responding tissues such as jejunal crypt mucosal [22,27,28] and skin [29]. Taxol and docetaxel alone were effective in arresting jejunal crypt cells in mitosis and inducing apoptosis. In the study of Mason et al. [27], at 10 and 40 mg/kg b.w., single dose mitotic effect was found to reach a peak more rapidly at 3–4 h and return to basal level between 9 and 10 h after taxol treatment. Whereas, the apoptotic response peaked at 9–12 h and decreased to basal levels 24 h after taxol treatment [22,27]. In our study, the mitotic index decreased in a dose-dependent manner and a dose-dependent increase in chromosomal aberrations and the damaged cell ratio at 48 h were observed. This observation is in contrast with the observation reported by Mason et al. [27], where the mitotic index peaked at 3–4 hours after treatment and the apoptotic index peaked at 9–12 hours. This may be due to differences between our study and the study of Mason et al. [27] in the basal mitotic index ratio and also in the delayed response attributed to differences in mice species and sex study ratio. On the other hand, we evaluated the chromosomal aberration and the bone marrow cells.

Radiation caused a decrease in mitotic index in a dose dependent manner after 48 hour of treatment. A dose-dependent increase in chromosomal aberration and damaged cell ratio was observed. Mason et al. [27] showed an increase in both the mitotic index and the apoptotic index at 12 h after radiation. When compared to the results of our study, early increase in mitotic index and apoptotic index may be due to the higher radiation doses (11.8 to 13.8 Gy) used by Mason et al. [27] study, since in our study radiation doses ranged from 0.25 to 1 Gy.

In combination treatment exposures, no elevation in mitotic index or damaged cell ratio was observed at 12, 24, and 48 h after taxol pretreatment in most of the combination groups when compared to radiation induced mitotic index and damaged cell ratio. In contrast to this observation, the majority of the combination groups showed higher damaged cell ratio when compared to the taxol induced damaged cell ratio. These observations strongly suggest that this is protective, since no prominent elevation in the TER was observed. This observation is in agreement with the studies reported by Mason et al. [27] and Milas et al. [22].

Recently there was some interest about the use of low dose radiation in combination with taxol [30]. In this study, the effect of fractionated low dose radiation in combination with taxol on the cells with wild (HCT-111) or mutant type p53 (HT-29) was investigated. Different radiation doses (0.6–6 Gy) or paclitaxel (1–10 nM) or both in combination were used. It was found that pretreatment with paclitaxel followed by multifractionated low dose radiation (0.5–1 Gy fractions for a total dose of 2 Gy) significantly enhanced the radiosensitizing effect in both HCT-116 and HT-29. In our study, pretreatment with taxol at 40 mg/kg b.w. followed by low doses radiation (0.25 and 0.5 Gy) showed a relatively small enhancement ratio (1.20 and 1.83, respectively).

These findings suggest that taxol may cause a radiosensitizing effect in bone marrow cells. Forty-eight hour pretreatment of taxol exposure followed by low dose radiation may cause induction of chromosomal aberrations when compared to other taxol timing sequences.
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