Effects of Taxol plus radiation on the apoptotic and mitotic indices of mouse intestinal crypt cells

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Abstract Purpose: In this study we investigated the effect of Taxol, radiation, or Taxol plus radiation on highly proliferative normal tissue – the intestinal crypt cells of Swiss albino mice. Materials and methods: Swiss-albino mice, 3–4 months old, were used in this study. Taxol was administered by bolus intravenously through the tail vein. Radiation was given using a linear accelerator. There were four treatment categories, which comprised a total of 34 groups. Each group consisted of five animals. The first treatment category was a control category which comprised one group (n = 5). The second treatment category was Taxol alone which comprised three groups (n = 15). The third treatment category was radiation alone which comprised three groups (n = 15). The fourth treatment category was Taxol plus radiation which comprised 27 groups (n = 135). Mice were killed 24 h after Taxol or radiation or combined administration using ether anesthesia. Using a light microscope, apoptotic and mitotic indices were counted on jejunal crypt cells of mice that were stained with hematoxylin-eosin. Differences between groups were statistically evaluated with Student’s t-test. Results: Taxol caused a dose-dependent increase in apoptosis (P = 0.045) and decreased the mitotic index (P = 0.006) at high doses. Similarly, radiation caused a dose-dependent increase in apoptosis (P = 0.046) and decreased the mitotic index (P = 0.299) at higher radiation doses. Compared to radiation alone, Taxol caused a significant induction of apoptosis (P = 0.010). In combination, no significant radiosensitizing effect of Taxol was observed (enhancement ratio < 1), when compared to radiation alone. However, an increase in apoptosis was observed after 24 h of Taxol exposure when compared to 12 or 48 h of Taxol exposure (P = 0.0001 and P = 0.0001). Conclusion: These findings suggest that Taxol did not cause a radiosensitizing effect in intestinal crypt cells. However, a 24-hour pretreatment of Taxol exposure followed by radiation caused significant induction of apoptosis and reduction of the mitotic index when compared to other Taxol timing sequences. Thus, the lack of a radiosensitizing effect of Taxol in these proliferative cells may be due to enhanced mitotic death rather than apoptotic death.

Key words Taxol · Radiation · Mitotic index · Apoptotic index · Intestinal crypt 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 the G2 and M phase by preventing polymerization of microtubules. It causes formation of abnormal microtubules and aggregation of microtubules (Foa et al. 1994; Rowinsky et al. 1990; Schiff et al. 1979; Schiff et al. 1980). Antitumoral effects of Taxol have been shown in several types of cancers such as ovarian, breast, lung, and head and neck cancers (Farrelltiere 1995; Holmes et al. 1991; McGuire et al. 1989; Murphy et al. 1993). It has also been demonstrated that Taxol is a potent radiosensitizer in human cancer cell lines (Choy et al. 1993; Cook et al. 1993; Hei
and Hall 1993; Hei et al. 1994; Liebmann et al. 1993; Liebmann et al. 1994a; Liebmann et al. 1994b; Liebmann et al. 1994c; Steren et al. 1993).

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 to radiation damage (Sinclair and Morton 1966; Terasima and Tolmach 1963; Withers 1974). It has been speculated that Taxol might sensitize cells to radiation damage by blocking them in the G2 and M phases (Liebmann et al. 1994a; Milas et al. 1995; Milas et al. 1996). In addition to its effect on the 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 (Liebmann et al. 1994a; Liebmann et al. 1994c; Milas et al. 1995).

Although all phases of the cell cycle are affected by stabilization of microtubules, the greatest effect is seen in mitotically active cells (Murphy et al. 1993; Rowinsky et al. 1990). Cells that are affected by a cell-cycle block or prolonged cytokinesis may undergo apoptosis or necrosis or can complete mitosis by the release of the cell-cycle block (Bhalla et al. 1993; Hruban et al. 1991; Milas et al. 1995).

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 (Hruban et al. 1991; Rowinsky et al. 1992). Although numerous papers have been published showing the increased apoptotic effect of combined Taxol and radiation exposure in animal tumors and in several cancer cell lines, there are few studies investigating the effects on normal tissue.

Thus, in this study, we exclusively investigated the effects of Taxol plus radiation on highly proliferating normal intestinal crypt 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 of tumors by sparing normal tissue.

Materials and methods

Animals

One hundred and seventy in-bred Swiss albino mice obtained from the Test Animals Breeding 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 g. 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 comprised a total of 34 groups. Each group consisted of five animals.

Treatment categories

The first was the control category which comprised one group (total n = 5). The second treatment category was Taxol alone which comprised three groups (total n = 15). The third treatment category was radiation alone which comprised three groups (total n = 15). The fourth treatment category was Taxol plus radiation which comprised 27 groups (total n = 135).

Taxol and irradiation treatment

Taxol was administered by bolus 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 the 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 intravenously at a dose of 10, 20 or 40 mg/kg, initially. They were then irradiated at a dose of 0.25, 0.5 or 1 Gy after 12-, 24- or 48-hour Taxol pre-treatment.

Analysis of the mitotic and apoptotic indices

Mice were killed 24 h after treatment administration using ether anesthesia. Intestines were dissected and 2 cm of jejunum was taken out and fixed with 10% neutral formalin. Three to five transverse sections were taken from each animal and embedded in paraffin blocks as per standard histological tissue procedures. Four-micrometer sections were stained with hematoxylin-eosin. Using a light microscope, the apoptotic and mitotic indices were counted on jejunal crypt cells of mice. Apoptotic bodies were identified as per the criteria suggested by Kerr et al. and mitotic indices were counted on jejunal crypt cells of mice. Apoptotic bodies were observed as structures with condensed, homogenous cytoplasm, and with dark, condensed chromatin. Condensed chromatin was seen in certain parts in some apoptotic bodies while cytoplasm was not readily observed in others. Certain apoptotic bodies were surrounded with a clear halo separating them from the neighboring cells.

Five hundred crypt cells were counted for each animal at five randomly selected fields by using x400 magnification. The rates of apoptotic and mitotic cells to the total number of cells were calculated and given as mitotic and apoptotic index values. Differences between groups were statistically evaluated with Student’s t-test.

Radiation enhancement ratio (RER) for Taxol was calculated using the formula defined by Chendil et al. (2000):

$$\text{RER} = \frac{\text{apoptotic index of radiation alone}}{\text{apoptotic index of radiation plus Taxol}}$$

Taxol enhancement ratio (TER) for radiation was calculated using the formula:

$$\text{TER} = \frac{\text{apoptotic index of Taxol alone}}{\text{apoptotic index of radiation plus Taxol}}$$

An RER or TER of >1 indicated radiosensitization or chemosensitization to Taxol or radiation, respectively.

Results

Low apoptotic index and mitotic index in intestinal crypt cells of control mice group

In the control group, five mice were used to obtain the baseline frequency of the mitotic and apoptotic indices. The results of this control group showed a mitotic index mean of 2.8 and an apoptotic index mean of 1. These results suggest a low frequency of mitotic and apoptotic indices in the untreated control group.