

## Interaction between $\beta$ -Lapachone and Ionizing Radiation

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### 1. Introduction

$\beta$ -Lapachone (3,4-dihydro-2,2-dimethyl-2H-naphtho[1,2-b]pyran-5,6-dione)( $\beta$ -lap) was originally isolated from the bark of the Lapacho tree growing in South America (1). This drug has attracted considerable interest in recent years because of its potent cytotoxicity against various cancer cell lines through a mechanism that works independent of the cell cycle of p53 status. Interestingly,  $\beta$ -lap has been reported to react synergistically with Taxol, mitomycin C, genistein, and ionizing radiation (IR) (2-3) *in vitro* against cultured cancer cells. It has also been reported that  $\beta$ -lap inhibits the repair of potentially lethal radiation damage by converting repairable single-stranded DNA breaks into repair-resistant, double-stranded DNA breaks. Thus  $\beta$ -lap has been thought to act as a radiation sensitizer by inhibiting DNA damage repair. In the present study, we observed that IR sensitizes cancer cells to  $\beta$ -lap. It thus appeared that the synergistic interaction of IR and  $\beta$ -lap in killing cancer cells was due to an increase in cellular susceptibility to  $\beta$ -lap, probably in addition to  $\beta$ -lap-induced radiosensitization.

### 2. Materials and Methods

#### 2.1 $\beta$ -Lapachone, cells and clonogenic survival assay

$\beta$ -lap was purchased from a commercial source (Sigma, St. Louis, MO). It was dissolved in dimethyl sulfoxide at 10 mM, diluted to desired concentration in RPMI 1640 medium, and used to treat cells with  $\beta$ -lap alone or in combination with IR *in vitro*. FSaII cells, a fibrosarcoma of C3H mice, were used. Cells were maintained in RPMI 1640 medium (GIBCO BRL, Grand Island, NY) supplemented with 10% bovine calf serum, penicillin (50 units/mL), and streptomycin (50  $\mu$ g/mL) at 37°C in a humidified 95% air-5% CO<sub>2</sub> atmosphere. The population doubling time of FSaII cells used in the present study was  $23 \pm 3$  h *in vitro*. For experiments, cells in exponential growth phase were dispersed to single cells by treatment with 0.25% trypsin for 10 min, washed twice with medium containing 10% bovine calf serum, and appropriate numbers of cells were seeded in 25 cm<sup>2</sup> plastic tissue culture flasks with 5 mL RPMI

1640 medium. After an overnight attachment period, the effects of  $\beta$ -lap alone or in combination with IR exposures were examined. For clonogenic survival assays, mock or experimentally treated cells were cultured for 7–8 days after treatment, and colonies were fixed with a mixture of methanol and acetic acid (10:1 v/v) and stained with 1% crystal violet. Colonies containing more than 50 cells were scored.

#### 2.2 Effect of $\beta$ -lap alone or in combination with radiation on tumor growth

Exponentially growing FSaII tumor cells in culture were harvested, washed, and suspended in serum-free RPMI medium. About 0.05 mL of the suspension containing  $2 \times 10^5$  tumor cells was injected subcutaneously into the right thighs of female C3H mice weighing 20–23 g. When tumors grew to 7–8 mm in diameter, a group of mice were intraperitoneally injected with 50 mg/kg  $\beta$ -lap dissolved in 0.2 mL of HP- $\beta$ -CD ( $\beta$ -hydroxypropyl- $\beta$ -cyclodextrin). A second group of mice bearing tumors of equivalent volumes were treated with 20 Gy X-irradiation in a single exposure. Finally, a third group of tumor-bearing mice received combined treatment: mice were injected intraperitoneally with  $\beta$ -lap at 50 mg/kg and the tumors were treated with 20 Gy X-irradiation 30 min after drug injection. The mice that did not receive  $\beta$ -lap injection received an injection of 0.2 mL of solvent, HP- $\beta$ -CD, only. For the irradiation of tumors grown in the thigh, mice were anesthetized with an intraperitoneal injection of a mixture of 100 mg/kg ketamine and 10 mg/kg xylazine. Except for the tumor area, the whole body of the anesthetized mice was covered with a 4-mm thick lead shield and the tumors were irradiated with 250-kV orthovoltage X-rays at 1.4 Gy/min. Control animals were mock-irradiated. Tumor diameters were measured with a caliper, and tumor volumes were calculated using the formula:  $V = a^2b/2$ , where  $a$  was the shortest tumor diameter and  $b$  was the longest tumor diameter measured.

### 3. Results

#### 3.1 $\beta$ -lap cytotoxicity against FSaII cells

Figure 1 shows changes in % survival of clonogenic FSaII cells treated with different concentrations of  $\beta$ -lap for varying lengths of time. When cells were incubated with 2.5  $\mu$ M  $\beta$ -lap, survival declined only slightly during the first 6 h, but decreased rapidly thereafter. In contrast, incubation of cells with 5  $\mu$ M  $\beta$ -lap for only 2h significantly decreased survival. The clonogenic cell survival decreased to 12.8% and 1.5% after incubation with 5  $\mu$ M  $\beta$ -lap for 4h and 6h, respectively. Incubation of cells with 10  $\mu$ M  $\beta$ -lap for 6 h resulted in less than 0.03% survival.

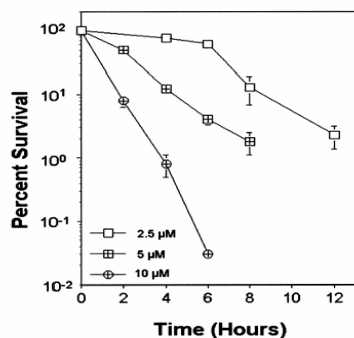


Figure 1. Cytotoxicity of  $\beta$ -lap on FSaII cells

### 3.2 Irradiation increases the $\beta$ -lap-induced clonogenic cell death

Figure 2 shows the survival curve of FSaII cells treated with various doses of IR in combination with a 4-h treatment of 5  $\mu$ M  $\beta$ -lap, applied either before or immediately after IR exposure. The Do of the radiation survival curve of control cells (radiation therapy only) was 125 cGy and that of the cells treated with  $\beta$ -lap for 4 h, washed, and irradiated was 123 cGy. This result indicated that the radiosensitivity of cells was not affected by prior treatment with  $\beta$ -lap. On the other hand, the Do of the radiation survival curve of cells treated with  $\beta$ -lap for 4 h immediately after radiation exposure was 82.5 cGy. It was concluded that  $\beta$ -lap treatment and IR reacted in a synergistic manner when  $\beta$ -lap treatment was applied after radiation exposure, whereas the two treatment regimens reacted additively when  $\beta$ -lap treatment was applied before radiation exposure. The effect of adding  $\beta$ -lap to cells immediately before IR and washed 4 h later (data not shown) was identical to the effect of treating the cells with  $\beta$ -lap for 4 h starting immediately after radiation exposure.

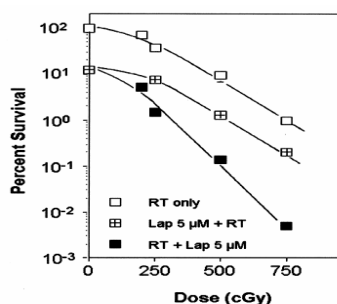


Figure 2. Survival curves of FSaII tumor cells treated with radiation alone or with  $\beta$ -lap treatment

### 3.3 Irradiation increases anti-tumor activity of $\beta$ -lap

Figure 3 shows changes in the volume of FSaII tumors after various treatments. An intraperitoneal injection of 50 mg/kg  $\beta$ -lap suppressed tumor growth and enhanced the effect of IR to suppress the tumor growth. The numbers of days required for a fourfold increase in mean tumor volumes for control,  $\beta$ -lap alone, IR alone, and IR+  $\beta$ -lap groups were  $5.3 \pm 0.2$ ,  $7.1 \pm 0.3$ ,  $11.1 \pm 0.5$ , and  $18.4 \pm 0.3$  days, respectively. Consequently, for the fourfold increase in tumor volume, the growth delay (difference between the control and treated groups) caused by  $\beta$ -lap alone, IR alone, and the combination of  $\beta$ -lap and IR treatment were 1.8 days, 5.8 days, and 13.1 days, respectively. The growth delay by  $\beta$ -lap alone was statistically significant ( $p < 0.001$ ), and the growth delay caused by  $\beta$ -lap plus IR was significantly longer than that caused by IR alone ( $p < 0.01$ ).  $\beta$ -lap treatment did not appear to increase the radiation-induced damage in normal tissues adjacent to the treated tumors, although the normal tissue damage was not quantitatively determined in the present study.

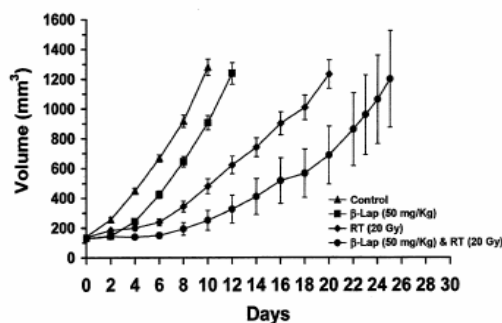


Figure 3. Difference of growth delay of FSaII tumors by  $\beta$ -lap alone, RT alone, and  $\beta$ -lap+RT

## 4. Conclusion

Ionizing radiation increases the cytotoxicity of Lapachone to cancer cells. Lapachone could be used as a potent radiosensitizer for killing cancer cells in solid tumors.

## REFERENCES

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