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Synergistic interaction between ionizing radiation and β-lapachone against cancer cells

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1. Introduction

B-lapachone (β-lap) is a quinone compound known to poses powerful anti-cancer effect. We have observed that ionizing radiation (IR) and β-lap kill cancer cells in a synergistic manner. It has been reported that β-lap is bio-activated by NAD(P)H:quinone oxidoreductase (NQO1) and thus the cytotoxicity of β-lap is dependent on NQO1 activity. We have previously reported that NQO1 is activated by various stresses including IR. The present study was undertaken to reveal the mechanisms underlying the synergistic interaction of β-lap with IR. In particular, we have investigated the relationship between the IR-induced activation of NQO1 and the sensitivity of cells to β-lap.

2. Materials and Methods

2.1 Effect of β-lap and irradiation on clonogenic cell survival
To evaluate the cytotoxicity of β-lap, using A549 human lung epithelial cancer cells. Cells were treated with β-lap for varying lengths of time at 37°C, gently rinsed twice with culture medium, and incubated at 37°C with complete medium for 8-9 days. Resulting colonies were fixed and stained with 1% crystal violet, and the numbers of colonies containing more than 50 cells were counted. For the study of radiation effects on clonogenic survival, cells were irradiated with a Cecium-137 irradiator and the clnogenic cell survival was determined. To determined the combined effect of irradiation and β-lap, cells were irradiatiated, maintained at 37°C for varying lengths of time and then treated with β-lap.

2.2 Effect of siRNAs-NQO1 on β -lap cytotoxicty[1]
siRNAs-NQO1 was purchased from Ambion, Inc. (Austin, TX, USA). The siRNAs was 21 nucleotides long and contained symmetric 3′ overhangs of two deoxythymidines. Transfection of siRNA-NQO1 was conducted according to the manufacturer's direction.. The sensitivity of the resultant siRNA-NQO1 transfected cells to β-lap was assessed by incubating the cells with 5 μM β-lap for 4 h and determining the clonogenic survival.

2.3 NQO1 enzyme activity
Cells were harvested by trypsinization, washed with PBS, phenol red-free Hank's balanced salt solution, then re-suspended in PBS, pH 7.2, containing 10 μg/ml aprotinin. Cell suspensions were sonicated and the NQO1 activity in the resulting S9 supernatants was determined with or without presence of 20 μM dicoumarol. The enzyme activity reduced by dicoumarol was taken as NQO1 activity [2,3].

3. Results and Discussion

3.1 Combined effect of β-lap and radiation on clonogenic cell survival
A 4 h incubation with 5 μM B-lap reduced the clonogenic cell survival to 9.2%. Figure 1 shows the radiation survival curve of A549 cells treated with radiation alone and that of the cells irradiated first and then immediately exposed to 5 μM β-lap for 4 h. The survival curve for the combined effect was normalized for the survival of the cells treated with β-lap alone. The survival curve became steeper when cells were treated with β-lap immediately after irradiation. In particular, the initial shoulder region of the radiation survival curve of the cells was steeper than that of cells received irradiation exposure alone. This result suggested that β-lap inhibits repair of sublethal radiation damage. As shown in Fig.1B, the clonogenic cell death caused by irradiation immediately followed by 4 h incubation with β-lap treatment was significantly greater than the cell death expected to occur if the two modalities acted merely additively (dotted line)($p<0.05$). The survival of cells irradiated and treated with β-lap treatment further decreased as the time interval between irradiation and β-lap treatment was increased to 4-24 h. It was evident that the irradiated cells were sensitive to β-lap treatment, indicating that radiation sensitized the cells to β-lap. In contrast, the combined effect was only slightly greater than additive when A549 cells were treated first with β-lap, washed and then immediately irradiated (data not shown), as we previously observed in FSaII mouse tumor cells [2,3].

3.2 *Effect of radiation on NQO1 expression and activity*
Both the immunostaining of cells (Fig.2A) and the Western blotting (Fig.2B) showed that the NQO1 expression markedly increased by 2 h after a 4 Gy irradiation and remained increased at almost the same level until 24 h after irradiation, the extent of our study. The NQO1 enzyme activity increased about 40% after 4

Gy irradiation and remained increased until 24 h after irradiation.

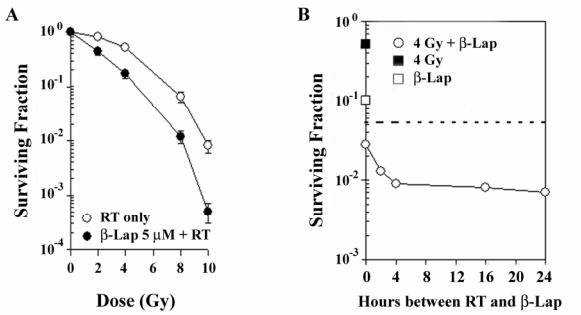

Figure 1. Combined effects of β-lap and radiation on the survival of A549 cells.

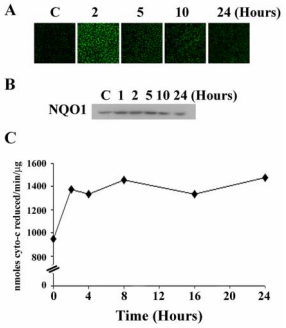

Figure 2. Effects of radiation on NQO1 expression and activity.

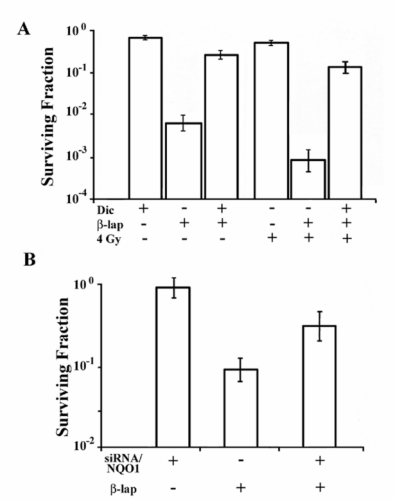

Figure 3. (A) Effects of dicoumarol on B-lap-induced clonogenic cell death
(B) Effects of sRNA-NQO1 on B-lap induced clonogenic cell death

3.3 Suppression of β-lap cytotoxicity by dicoumarol or siRNA-NQO1

A 4 h incubation with 50 μM dicoumarol reduced the clonogenic cell survival to 73.8 ± 6.2 % and that a 4 h incubation with 10 μM β-lap decreased the clonogenic survival to 0.51 ± 0.18 %. On the other hand, when cells were incubated with 10 μM β-lap together with 50 μM dicoumarol for 4 h, the cell survival decreased to 25.9 ± 2.9 %, which was about 50-fold greater than the cell survival after treatment with β-lap alone ($p<0.05$). The treatment of cells with 4 Gy irradiation immediately followed by a 4 h incubation with 10 μM β-lap reduced cell survival to 0.09 ± 0.05%, but the cell survival increased to 14.5 ± 3.0 %, a 14-fold increase ($p<0.05$), when the irradiated cells were incubation with 10 μM β-lap together with 50 μM dicoumarol. Figure 3B shows the effects of siRNA-NQO1 on the β-lap-induced cell death. The siRNA-NQO1 alone exerted no effect on the clonogenic survival of cells. Like dicoumarol, siRNA-NQO1 reduced the cytotoxicity of β-lap as demonstrated by the significantly increased cell survival ($p<0.05$) when cells were transfected with siRNA-NQO1 prior to β-lap treatment as compared with the survival of cells treated with β-lap alone. These results clearly demonstrated that NQO1 plays a cardinal role in the B-lap-induced cell death and radiosensitization.

4. Conclusion

We have investigated the effect of β-lapachone (β-lap), an experimental anti-cancer drug, in combination with ionizing radiation against A549 human lung epithelial cancer cells. A 4 h treatment with 5 μM β-lap caused rapid apoptosis and clonogenic cell death in a dose dependent manner. The cytotoxicity of β-lap could be markedly reduced by dicoumarol, an inhibitor of NAD(P)H:quinone oxidoreductase (NQO1), and also by si-RNA of NQO1 indicating that bioreduction of β-lap mediated by NQO1 is an important step in β-lap-induced cell death. In addition to causing apoptosis and clonogenic cell death, β-lap treatment applied immediately after irradiation sensitized cells to radiation by inhibiting repair of sublethal radiation damage. It was therefore concluded that radiation potentiated the response of cells to β-lap by increasing NQO1 activity. In summary, radiation and β-lap synergistically kill cancer cells through two distinct mechanisms: first, β-lap sensitizes cells to radiation by inhibiting repair of radiation damage, and second, radiation sensitizes cells to β-lap by causing long-lasting upregulation of NQO1.

Acknowledgements

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