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Synergistic effect of ionizing radiation and β-lapachone against tumor in vitro and in vivo

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β-lapachone(β-Lap), a natural o-naphthoquinone, presents in the bark of the Lapacho tree. β-Lap is cytotoxic against a variety of human cancer cells and it potentiates the anti-tumor effect of Taxol. In addition, β-Lap has been reported to radiosensitize cancer cells by inhibiting the repair of radiation-induced DNA damage.In the present study, we investigated the cytotoxicity of β-Lap against RKO human colorectal cancer cells as well as the combined effect of β-Lap and ionizing radiation. An incubation of RKO cells with 5 μM of β-Lap for 4 h killed almost 90% of the clonogenic cells. An incubation of RKO cells with 5 μM of β-Lap for 4 h or longer also caused massive apoptosis. Unlike other cytotoxic agents, β-Lap did not increase the expression of p53 and p21 and it suppressed the NFkB expression. The expression of Caspase 9 and 3 was minimally altered by β-Lap. Radiation and β-Lap acted synergistically in inducing clonogenic cell death and apoptosis in RKO cells when β-Lap treatment was applied after but not before the radiation exposure of the cells. Interestingly, a 4 h treatment with 5 μM of β-Lap starting 5 h after irradiation was as effective as that starting immediately after irradiation. The mechanisms of β-Lap-induced cell killing is controversial but a recent hypothesis is that β-Lap is activated by NAD(P)H: quinone-oxidoreductase (NQO1) in the cells followed by an elevation of cytosolic Ca^{2+} level and activation of proteases leading to apoptosis. It has been reported that NQO1 level in cells is markedly up-regulated for longer than 10 h after irradiation. Indeed, using immunological staining of NQO1, we observed a significant elevation of NQO1 expression in RKO cells 5h after 2-4 Gy irradiation. Such a prolonged elevation of NQO1 level after irradiation may be the reasons why the β-Lap treatment applied 5 h after irradiation was as effective as that applied immediately after irradiation in killing the cells. In view of the fact that the repair of radiation-induced damage is usually completed within 1-2 h after irradiation, it is highly likely that the β-Lap treatment applied 5 h after irradiation could not inhibit the repair of radiation-induced damage. For in vivo study, RKO cells were injected S.C. into the hind-leg of Nu/Nu mice, and allowed to grow to 130 mm3 tumor. The mice were i.p. injected with β-lapachone or saline 2 h after irradiation of tumors with 10 Gy of X-rays. The radiation induced growth delay was increased by 2.4 μg/g of β-lapachone. Taken together, we may conclude that the synergistic interaction of radiation and β-Lap in killing cancer cells is not due to radiosensitization by β-Lap but to an enhancement of β-Lap cytotoxicity by radiation through an upregulation of NQO1. The fact that NQO1 is elevated in tumors and that radiation causes prolonged increase of the NQO1 expression may be exploited to preferentially kill tumor cells using β-Lap in combination with radiotherapy.