Resveratrol and piperine enhance radiosensitivity of tumor cells

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The use of ionizing radiation (IR) is essential for treating many human cancers. However, radioresistance markedly impairs the efficacy of tumor radiotherapy. IR enhances the production of reactive oxygen species (ROS) in a variety of cells which are determinant components in the induction of apoptosis. Much interest has developed to augment the effect of radiation in tumors by combining it with radiosensitizers to improve the therapeutic ratio. In the current study, the radiosensitizing effects of resveratrol and piperine on cancer cells were evaluated. Cancer cell lines treated with these natural products exhibited significantly augmented IR-induced apoptosis and loss of mitochondrial membrane potential, presumably through enhanced ROS generation. Applying natural products as sensitizers for IR-induced apoptotic cell death offers a promising therapeutic approach to treat cancer. [BMB reports 2012; 45(4): 242-246]

INTRODUCTION

Ionizing radiation (IR) has long been used to treat patients with cancer (1). However, dose-limiting normal tissue toxicity and radioresistant tumors are still linked to life-threatening radiation treatment failure. Much interest has developed to augment the effect of radiation on tumors by combining it with targeted tumor therapeutics to improve the therapeutic ratio of these two factors (2). Therefore, efforts of many researchers have been focused on radiosensitizers, which lower the radiation dose-response threshold for cancer cells without enhancing the radiosensitivity of normal cells (1, 3).

The induction of apoptosis in cancer cells has become an indicator of the cancer treatment response and reduced mortality in patients with cancer (4). IR enhances ROS generation in a variety of cells (5). ROS, such as superoxide, hydroxyl radicals, singlet oxygen, and hydrogen peroxide, damage critical cellular components such as DNA, proteins, and lipids, eventually causing physical and chemical damage to tissues that subsequently leads to apoptosis or neoplastic transformation (6). Because ROS are responsible for triggering cell death induced by IR, the production of additional ROS leads to irreversible oxidative stress (7). A unique antitumor strategy named oxidative therapy or pro-oxidant cancer therapy has been developed by inducing ROS generation directly on tumor cells or by preferentially inhibiting the antioxidative defense systems of tumor cells (7-9).

Here, the radiosensitizing effects of two natural products on tumor cells were investigated. Several natural products act as antioxidants in normal cells but are prooxidants in cancer cells. Resveratrol, a polyphenol found in berries, nuts, and red wine, has a variety of cancer chemopreventive activities including anti-inflammatory, pro-apoptotic, anti-angiogenic, and chemosensitizing properties in a variety of cultured cells and in vivo systems (10). Although resveratrol is redox active and has been claimed to be an antioxidant (11), reports also show the pro-oxidant capacity of resveratrol (12, 13). Piperine, a main component of Piper longum Linn. and Piper nigrum Linn., is a plant alkaloid with a long history of medicinal use. Piperine exhibits a variety of biological activities, including anti-pyretic, anti-inflammatory, anti-depressant, hepatoprotective, and antitumor activities (14).

The results of this study showed that resveratrol and piperine enhanced the radiosensitivity of cancer cell lines by increasing ROS generation. Applying natural products as sensitizers for IR-induced apoptotic cell death offers a promising therapeutic approach for treating cancer.

RESULTS AND DISCUSSION

ROS initiate several cellular signal transduction pathways that may either aid the cell in coping with the excess oxidative stress resulting from the IR or activate pathways that lead to cell destruction beyond repair (15, 16). Naturally occurring compounds that enhance ROS production in cancer cells under IR may be developed as promising radiosensitizers from the perspective of the cancer cell killing potential of ROS. In the present study, the sensitizing effects of resveratrol and piperine on IR-induced apoptosis were evaluated.
Enhanced cell death and a sensitizing effect of resveratrol and piperine on apoptosis in cancer cells were observed when cultured mouse colon carcinoma CT26 (Fig. 1) and mouse melanoma B16F10 cells (Fig. 2) were exposed to γ-irradiation. Previous studies have identified caspases as important mediators of apoptosis induced by a range of apoptotic stimuli (17). Cleavage of procaspase-3 induced by IR was more pronounced in cells treated with the natural products. Cleaved poly (ADP-ribose) polymerase products increased markedly when the cells were exposed to the combination of IR and the natural products compared to those in either treatment alone. The abundance of Bcl-2, an anti-apoptotic protein, decreased significantly following the combined treatment of IR and the natural products. Bid, a death agonist member of the Bcl-2/ Bcl-X family, is a specific proximal substrate of caspase-8 in the Fas signaling pathway (18). When cells were exposed to IR, Bid cleavage was enhanced in cells treated with natural products compared to that in untreated cells.

Alterations in mitochondrial integrity and function may play an important role in the apoptotic cascade. The mitochondrial membrane potential (MMP), associated with the opening of large pores in mitochondrial membranes, is a very important event in apoptosis, and ROS are one of the major stimuli that alter the MMP (19). The change in the MMP was assessed by measuring the intensity of fluorescence emitted from the lipophilic cation dye rhodamine 123 to evaluate whether the natural products modulated the MMP upon exposure to IR. In this assay, high fluorescence indicates healthy mitochondria. Significantly less rhodamine 123 dye was taken up by the mitochondria of cells treated with natural products compared to