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Effect of Pentoxifylline on Radiation-Induced G_2-Phase Delay and Radiosensitivity of Human Colon and Cervical Cancer Cells

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Cells of three adherent cell lines with mutated p53 (WiDr and C33-A) and disrupted p53 (C4-I) were used to investigate the effect of pentoxifylline (PTX) on radiation-induced G_2-phase block and its relationship to radiosensitivity. Postirradiation exposure to 0.25–1.0 m*M* PTX resulted in an increase in radiosensitivity in a concentration-dependent manner as determined by a clonogenic assay. The change in radiation sensitivity was quantified by calculating the enhancement ratio (ER) at a clinically relevant dose of 2 Gy; the ER for WiDr cells was 1.23 ± 0.03 and 1.39 ± 0.15 for 0.5 and 1.0 m*M* PTX, respectively. For C33-A cells, the ER ranged from 1.04 ± 0.04 to 1.99 ± 0.17 for 0.25–1.0 m*M* PTX, whereas for C4-I cells the values were 1.29 ± 0.04 and 1.76 ± 0.17 for 0.25 and 0.5 m*M* PTX. In asynchronous WiDr, C33-A and C4-I cells, flow cytometry analysis showed a dose-dependent accumulation of cells in G_2/M phase which was detectable at 6 h and peaked at 12 h after irradiation. Such a G_2/M-phase block was transient at a dose of 2 Gy and persisted at 48 or 72 h after a dose of 4 or 6 Gy. At 12 h after 2 Gy, PTX significantly reduced the radiation-induced G_2/M-phase block in a dose-dependent manner. After the higher doses of 4 and 6 Gy, the dose-dependent G_2-phase arrest was significantly alleviated at 24 h by treatment with PTX, and the kinetics of this alleviation depended on the radiation dose. The results demonstrate that human colon and cervical cancer cells characterized by a mutated or disrupted p53 (i.e. not transfected) are radiosensitized by PTX, which alleviates the postirradiation G_2/M-phase block.

INTRODUCTION

Pentoxifylline (PTX), which is used widely in the treatment of intermittent claudication and peripheral vascular disease, has been demonstrated to potentiate the cytotoxic effects of various chemotherapeutic drugs (*1*, *2*). More recently, PTX has been investigated as a potential modifier of the effects of radiation (*2–6*). Enhancement of radiation-induced cytotoxicity has been demonstrated by postirradiation treatment with PTX *in vitro*, especially in cells with transfected mutated p53 (*7–12*). On the other hand, PTX has been shown to protect normal tissues against radiation damage (*13*), and it is also known as a drug that can improve tumor perfusion and hence oxygen supply (*6*, *8*, *14*).

The mechanism by which PTX may exert radiosensitization *in vitro* is suppression of repair of potentially lethal DNA damage by alleviation of the radiation-induced G_2/M-phase block (*3*, *12*). Recently, caffeine and its derivatives have been demonstrated to sensitize p53-deficient cells preferentially to DNA-damaging agents. Those cells were transfected with either mutant p53 or the human papilloma virus (HPV) *E6* gene and are characterized by the absence of a postirradiation G_1-phase block but persistence of the G_2-phase block as a consequence of loss of p53 function (*1*, *3*, *7*, *12*). In humans, most primary tumors have mutated p53 (*15*), and cervical carcinoma is frequently associated with HPV infection (*16*). Therefore, pharmacological agents such as caffeine and PTX may offer a means for selective enhancement of radiation sensitivity. Furthermore, the cytokinetic effect of PTX on radiation-induced G_2-phase block is not well elucidated (*3*, *7*, *10*, *17–19*). In the present study, cells of adherent cell lines with mutated p53 (WiDr and C33-A) and *E6*-disrupted p53 (C4-I) were used (*20–22*). We intended to investigate whether PTX enhances the cell killing induced by radiation. In addition, we analyzed the distribution of irradiated cells treated with PTX in the phases of the cell cycle to determine whether radiosensitization by PTX could be explained by alleviation of the G_2-phase block and whether there is any indication of a specific time course.

METHODS AND MATERIALS

Cell Culture and Chemicals

The human colon (WiDr) and cervical (C33-A) cancer cells were obtained from the American Type Culture Collection (Rockville, MD). The C4-I cervical cancer cells were a gift from Dr. P. Beard of the Swiss Institute for Cancer Research, Lausanne. WiDr cells were maintained in minimum essential medium containing 10% fetal bovine serum (FBS), 1% nonessential amino acids and 2 m*M* L-glutamine. C4-I cells were maintained in Dulbecco's modified Eagle's medium with 10% FBS. C-33A cells were maintained in Eagle's minimal essential medium with 10% FBS, 1% nonessential amino acids and 1% sodium pyruvate. The cells were incubated in a humidified 5% CO_2 atmosphere at 37°C. Under these conditions, the mean doubling time was 20–24 h for all the cells.