Study of the radiotherapy sensitization effects and mechanism of capecitabine (Xeloda) against non-small-cell lung cancer cell line A549

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ABSTRACT. The purpose of this study was to explore the radiotherapy sensitization effects and the mechanism of capecitabine (Xeloda) against the non-small-cell lung cancer cell line, A549**.** γ-[^{60}Co] radiation was used as the intervention method. Proliferative inhibition of capecitabine on A549 cells was determined by the CCK-8 method. The effects of capecitabine on the apoptosis rate and cell cycle distribution of A549 were detected with the flow cytometric method. We found that capecitabine inhibited the proliferation of A549 in a dose-dependent manner, notably increased the cell apoptosis rate and blocked the cellular G0/G1 phase after radiotherapy by γ-[^{60}Co]. Therefore, capecitabine can significantly increase the radiosensitivity of A549; its mechanism may be related to cell cycle arrest and induction of apoptosis.

Key words: Capecitabine (Xeloda); Non-small-cell lung cancer cell line A549; Radiation sensitive; Apoptosis

INTRODUCTION

Many cancer patients worldwide have received radiotherapy to control tumor growth. Although great technological progress has been made in radiation dose control, optimal radiation doses still cannot be used on some tumors owing to the risk of damage to normal tissues. One effective means of solving this problem is enhancing the radiosensitivity of tumor cells. Capecitabine (Xeloda) has been widely used in the radiotherapy of digestive tract cancer (Hameed and Cassidy 2011; Miger et al., 2014). There are fewer reports on its effects in the radiotherapy of non-small-cell lung cancer. In this study, non-small-cell lung cancer cell line A549 was treated with different concentrations of capecitabine to observe the cytotoxicity and radiosensitization of capecitabine and investigate its possible mechanism, which could provide the experimental basis for radiotherapy of non-small-cell lung cancer with capecitabine.

MATERIAL AND METHODS

Cell lines and reagents

Non-small-cell lung cancer cell line A549 was obtained from American Type Culture Collection. The cells were grown in RPMI1640 supplemented with penicillin, streptomycin, and 10% fetal bovine serum (Gibco-China, Shanghai, China). CCK-8 kit was purchased from Sigma-China (Shanghai, China). Annexin V-FITC/PI apoptosis detection kit was purchased from Becton-Dickinson Company (Franklin Lakes, NJ, USA).

Detection of cellular growth inhibition rate with CCK-8 kit

A549 cells taken from the logarithmic growth phase in good condition were inoculated in a 96-well plate, each well containing 3 x 10^3 cells. The culture medium was discarded after culture for 24 h and 100 µL of capecitabine at different concentrations (0, 25, 50, 100, 250, and 500 µM) diluted with RPMI1640 medium. The growth inhibition rate was detected with CCK-8 kit according to the manual. Briefly, CCK-8 (1:100) was added to the wells after treatment with capecitabine for 24, 48, and 72 h, respectively, and the wells were cultured continuously for 4 h. The absorbance at 490 nm wavelength was measured and the growth inhibition rate was calculated.

Determination of cellular apoptosis

A549 cells taken from the logarithmic growth phase in good condition were inoculated in 6-well plate, each well containing 2 x 10^5 cells. They were treated with 100 µM capecitabine for 24 h, with γ-[^{60}Co] 8Gy radiation for 6 h, and then washed twice with PBS. The cells were digested with 0.25% pancreatin:PBS = 1:1 digestive solution. The digested cells were centrifuged at 1000 r/min for 5 min and the supernatant was discarded. The collected cells were washed twice with RPMI1640. The apoptosis was determined with Annexin V-FITC/PI Apoptosis Detection Kit according to the manual. AnnexinV/PI was added to the cells, which were then incubated at room temperature for 15 min, avoiding light. The apoptosis was determined with flow cytometry.

Detection of cell cycle distribution

After treatment with capecitabine and radiation, the cells were collected by centrifugation. The cells were washed twice with PBS and re-suspended with pre-cooling PBS; pre-chilled ethanol was added and incubated on ice for 5 h. After washing, 1% ethidium iodide containing RNA enzyme was added for 30 min to stain the cells. The cell cycle distribution was detected with flow cytometry.

Statistical analysis

The experimental data were expressed as (Mean ± SD). All statistical analysis was done using SPSS 18.0 for Windows (IBM SPSS, Chicago, IL, USA.). The results were analyzed with one-way ANOVA and t-test to determine the significant differences. $P < 0.05$ was considered statistically significant.

RESULTS

Effects of capecitabine on the proliferation of A549 cells

A549 cells were treated with 25, 50, 100, 250, and 500 μM capecitabine. Their inhibition rates were 2.4 ± 3.3, 6.8 ± 2.1, 13.6 ± 4.2, 28.4 ± 3.8, and 40.5 ± 4.0%, respectively, after treatment for 24 h; after 48 h, the inhibition rates were 6.5 ± 3.2%, 11.4 ± 3.5, 18.2 ± 3.1, 37.1 ± 3.8, and 57.2 ± 4.4% respectively; and after treatment for 72 h, the inhibition rates were 17.6 ± 2.4, 28.8 ± 2.1, 39.3 ± 3.3, 58.8 ± 4.5, and 74.7 ± 5.8%, respectively. There were statistically significant differences in the inhibition rate between groups with different drug concentrations and different action time ($P < 0.05$). The growth inhibition curve is shown in Figure 1.

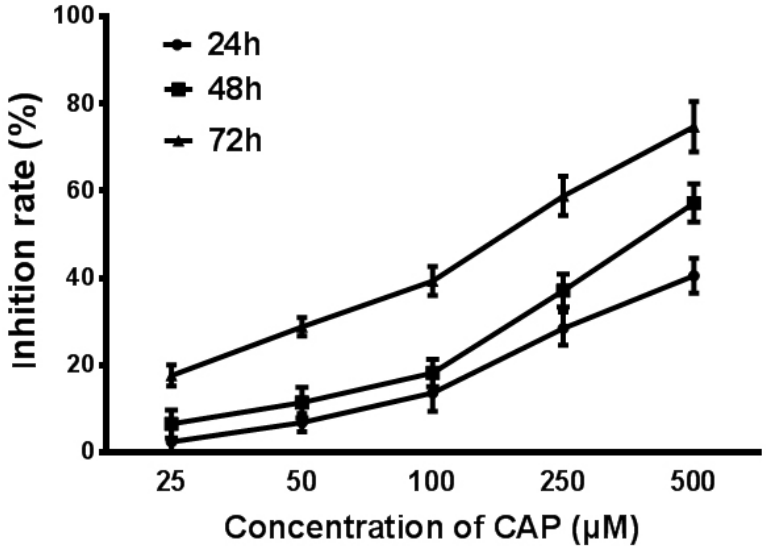

Figure 1. Inhibitory activity of different concentrations of capecitabine on the proliferation of A549 cells.

Detection of the effect of capecitabine on the apoptosis of A549 cells after radiotherapy with flow cytometry

The results of flow cytometry showed that A549 cells had a low apoptosis rate when treated with capecitabine; however, apoptosis could be induced. Compared with the control group, the apoptosis rate of the radiotherapy group increased obviously, and the apoptosis rate of the group pretreated with capecitabine prior to radiation was significantly higher than that of the radiotherapy group. Results are shown in Figure 2.

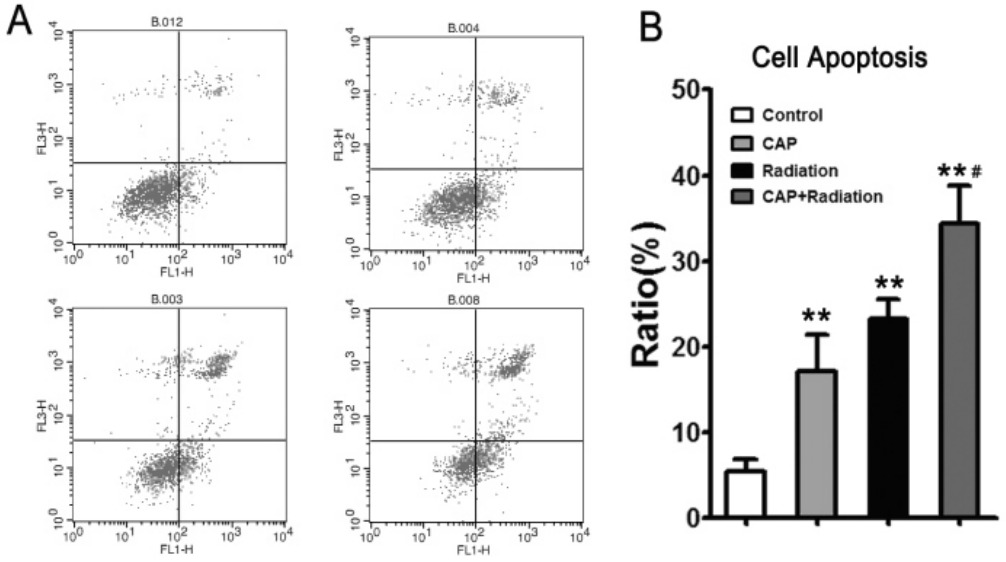

Figure 2. Effects of capecitabine on the apoptosis of A549 cells after radiotherapy. **P < 0.01 *vs* Control, #P < 0.05 *vs* radiation.

Detection of the effect of capecitabine on the cell cycle of A549 cells after radiotherapy with flow cytometry

The cell cycle distribution of A549 cells changed significantly after radiotherapy. When compared with the control group, the ratio of cells in the G0/G1 phase increased and the ratio of cells in the S phase decreased significantly in the capecitabine group, radiotherapy group, and capecitabine combined with radiotherapy group. Pretreatment with capecitabine significantly inhibited the cell cycle G0/G1 phase of A549 after radiotherapy. These results are shown in Table 1.

Table 1. Effects of capecitabine on cell cycle of A549 cells after radiotherapy.

Group	G0/G1	S	G2/M
Control	21.3 ± 1.21	51.4 ± 0.38	27.3 ± 1.45
CAP	52.1 ± 0.63*	29.7 ± 1.86*	18.2 ± 1.14
Radiation	44.7 ± 1.09*	37.8 ± 0.97*	17.5 ± 0.83
CAP+ radiation	72.5 ± 1.24*#	16.4 ± 1.06*#	11.1 ± 1.41

*P < 0.05 *vs* control, #P < 0.05 *vs* radiation.

DISCUSSION

Worldwide, lung cancer is one of the most common malignant tumors, with non-small-cell lung cancer (NSCLC) accounting for about 85% (Siegel et al., 2012). At present, effective therapy is very limited and the mortality rate from NSCLC is high, which seriously affects human health. With the continuous advancements in radiotherapy, it has been reported that total-dose radiation therapy can improve the survival rate of patients with non-small-cell lung cancer (Suntharalingam et al., 2012) and high-dose hypofractionated proton beam radiation therapy is safe and effective for central and peripheral early-stage non-small-cell lung cancer (Aumont-le et al., 2011; Bush et al., 2013). However, in the actual clinical practice, it was found that lung, liver, intestine, kidney, and spinal cord cancers were tolerant to radiation (Jansen et al., 2007). Tolerance to radiation has become one of the most challenging problems in cancer therapy. Cells undergo a complicated pathophysiological process after receiving radiation, involving abnormal activation of multiple signaling pathways (Jung et al., 2010; Xu et al., 2011), DNA damage of genes, the abnormal expression of proteins with the capacity of promoting DNA repair (Kasid et al., 1987; Kitahara et al., 2002; Fukuda et al., 2004), angiogenesis (Magnon et al., 2007), autophagy (Chaachouay et al., 2011; Zhuang et al., 2011), and apoptosis (Silva et al., 2014). Although these findings help to raise awareness and understanding of the cell radiosensitivity, the mechanism of this process is not clear.

Conventional chemotherapeutic agents include etoposide, platinum agents, anthracyclines, streptozotocin, and 5-fluorouracil (5-FU) (Jensen and Delle 2011). 5-FU is the first radiotherapy sensitizer used in clinical practice; its mechanism is thought to be inhibition of DNA synthesis by inhibition of thymidylate synthase (Tano et al., 2013). Others have reported that 5-FU could significantly inhibit the proliferation of A549 cells and prolong the cell cycle (Ham et al., 2013), and induce the apoptosis of PANC-1 cells (Lee et al., 2013). Capecitabine has been widely used in the radiotherapy of gastrointestinal cancer and breast cancer as an oral 5-FU pro-drug (Zhou et al., 2013; Saif 2014; Wang et al., 2014). In this study, we found that capecitabine could obviously inhibit the growth of A549 with dose and time dependence. Pretreatment with capecitabine significantly inhibited the cell cycle G0/G1 phase of A549 after radiotherapy. Capecitabine could induce cellular apoptosis. Both capecitabine and radiotherapy could cause cell arrest in the G0/G1 phase.

Capecitabine had obvious inhibitory effects on A549 cells. Its mechanism may be related to the changed cell cycle distribution and the increased sensitivity to radiotherapy that it induces. These could provide new ideas for the treatment of non-small-cell lung cancer.

Conflicts of interest

The authors declare no conflict of interest.

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