Increased radiosensitivity of human nasopharyngeal carcinoma cells by coixenolide under hypoxic condition

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Abstract Objective: To study the effect of coixenolide (CXL) on irradiation of human nasopharyngeal carcinoma cell line CNE-2Z in the absence of oxygen. Methods: Microcolony formation assay was used for determining the sensitivity of CNE-2Z to γ-ray in vitro. Results: When CNE-2Z was treated with $10^{-7} \sim 10^{-6}$ mol/L of CXL, CXL shifted the radiation dose-survival curve to the left, with the decrease of D_0, D_q and N values. Radiation dose reduced 10.07%~35.69% at D_{37} level, the sensitizing enhancement ratios (SER) were 1.07~1.43 and 1.16~1.72 at D_0 and D_q levels respectively. Conclusion: CXL increase susceptibility of CNE-2Z to ray under hypoxic condition by the mechanism of inhibiting its sublethal damage repair. Subject words Nasopharyngeal neoplasm; Coixenolide; Radiosensitization; Hypoxic irradiation

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1 Introduction

Coixenolide (CXL) is a component extracted from Chinese herbal drug coix seed, and has been reported to exert anticancer and immunoregulation actions[1]. We have found that CXL could enhance radiosensitivity of human nasopharyngeal carcinoma cell line CNE-2Z in the presence of oxygen (unpublished data). But what is more important clinically is whether CXL has the same effect under hypoxic condition.

1.1 Cell line

Cell line CNE-2Z (established in Guangdong medical college) was derived from poorly differentiated squamous cell carcinoma of human nasopharynx.

1.2 Drugs and reagents

CXL injection (Zhejiang Kanglaite pharmaceutical Co., LTD. Lot No. 98060092-2), RPMI1640(Gibco), calf serum (provided by the department of immunology, Sun Yat-sen university of medical science).

1.3 γ-Ray source

^{60}Co radiotherapy machine (ATC,USA)

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1.4 Methods

Microcolony formation assay[2] was used for determining the radiosensitivity of CNE-2Z.

CNE-2Z cells in the phase of logarithm growth were planted in 96-well microplate in which each well contains 0.2ml of cell suspension (200 cells). Medium was RPMI 1640 with 15%(v/v) of calf serum. CNE-2Z forming colonies were observed and counted with inverted microscope (Olympus IMF-2) 72 h after routine culture in CO_2 incubator. Each colony consisted of 8 or more cells, with clear-cut contour, powerful dioptric property and unanimous cell appearance in morphology.

The experiment of irradiation was made after CNE-2Z was incubated for 16 h. CNE-2Z was irradiated with a single dose of 1~13 Gy in the absence of oxygen, and then culture was kept on for 72h. Based on forming colony, survival fraction of CNE-2Z was calculated (=irradiated colony numbers/unirradiated colony numbers). Figure was drawn in logarithm of survival fraction vs radiation dose. The radiation dose-survival curve of CNE-2Z (without drug treatment) was regarded as control. In test groups, CNE-2Z was treated with CXL at final concentrations of $10^{-7} \sim 10^{-6}$ mol/L, then irradiated in the same manner, in order to investigate the influence of the drug on radiation dose-survival curve. Each dose point of radiation in each group contained 24 wells. In one culture plate planted with CNE-2Z, total was 4 groups that received a single dose of ray, one of them was control and the rest were tested with CXL treatment. Simultaneously there was an unirradiated group (24 wells) with hypoxic treatment for numerating survival fraction. Hypoxic device[3] is as follows. A box was made with perspex, two valves at both side of bottom controlled gas passing into and out respectively. Culture plate was put on the supporter in the bottom and the lid of the box was closed tightly with screw. In this way, the box was kept completely closed. Pure nitrogen (99.999%) flowed into the box through inlet valve and out through outlet valve, with a rate of about 0.5 L/min. 30 min after ventilation the two valves were shut down at the same time and irradiation was begun at room temperature. The influence of perspex box lid on ray was neglected. Radiation field was slightly larger than plate area, i.e. 9×13 cm. Depth was 5 cm. The distance from ^{60}Co source to cell layer was 80 cm, to perspex box surface was 75 cm. Dose rate was 1.0 Gy/min.

Besides, some other experiments, with three groups including group of radiation alone(C), group of CXL-treated before radiation (T_1) and group of CXL-treated after radiation (T_2), were performed for observing the influence of the time and order of the drug addition on radiation effect. Each group was with 24 wells. CXL was administered to T_1 at final concentration of 10^6 mol/L, with no drug to C and T_2. After incubation of 16 h, all groups were irradiated with a single dose of 3 Gy under hypoxic condition, then T_2 was treated with the same dose of CXL, with no drug to C and T_1. Culture was continued by the above method following irradiation and the colony formation of CNE-2Z was observed.

1.5 Data analysis and statistics

The parameters related to radiation dose-survival curve of CNE-2Z were analyzed with the equation $S=1-(1-e^{-D/D_0})^N$. S designates survival fraction. D stands for radiation dose. D_0 refers to the dose that made S in the linear part of the radiation dose-effect curve decreased 63%. N represented the interception in Y axial where the line is extended, is called extrapolate value. e was the basic number of natural logarithm. D0 means cell sensitivity to ray. N reflects the ability of cell repair to sublethal damage. The radiation dose-survival curve in this study showed that the exponential curve had a "shoulder". D_q denotes the threshold dose, and also relates to sublethal damage repair. These parameters were calculated using the methods reported in references[4,5]. The sensitizing enhancement ratio (SER) equals to D_0 or D_q value of control group/D_0 or D_q value of test group. In addition, dose reduction rate (DDR) of ray was numerated at D_{37} level. D_{37} is described as the radiation dose that causes S to reduce from 100% to 37%, namely $D_{37} = D_0 + D_q$, DRR(%)=[1-(D_{37} value of test group/D_{37} value of control group)]× 100%. Significant difference was compared using t or F test. All experiments were repeated 6 times (n=6).

2 Results

The rate of colony formation of CNE-2Z cells that underwent hypoxic treatment was 54.35±11.25% ($\bar{x} \pm s$, n=6, the same expression in the following parts). Administration of CXL (10^{-7}, $10^{-6.5}$ and 10^{-6} mol/L) was without influence on the proliferation of these cells. The rates were 58.12±12.71%, 61.42±13.75% and 52.33±10.95%, respectively. Variance analysis showed F=0.65, p >0.05 in comparison with the control group. After irradiation, CNE-2Z colony formation was suppressed (S drop), which exhibited positive correlation with the dose. Addition of CXL shifted the radiation dose-survival curve of CNE-2Z to the left (Fig.1), with decreased of D_0, D_q and N values (Table 1) and increased SER and DRR (Table 2). With combination of ray and CXL, treatment of CNE-2Z with the drug (10^{-6} mol/L) before or after irradiation (3 Gy) caused 32.73±7.12% and 28.56±5.83% of inhibition individually. A comparison between the two groups showed t = 1.109 <1.372, p >0.2.

Table 1 Influence of CXL on the parameters related to radiation dose-survival curve ($\bar{x} + s$, n=6)

	D_0/Gy	D_q/Gy	N
Radiation alone (control)	3.85±0.82	3.46±0.76	2.46±0.57
Radiation plus CXL (test)			
10^{-7} mol/L	3.60±0.94	2.98±0.69	2.296±0.61
$10^{-6.5}$ mol/L	3.11±0.73	2.34±0.56	2.12±0.42
10^{-6} mol/L	2.68±0.52	2.02±0.39	2.12±0.49

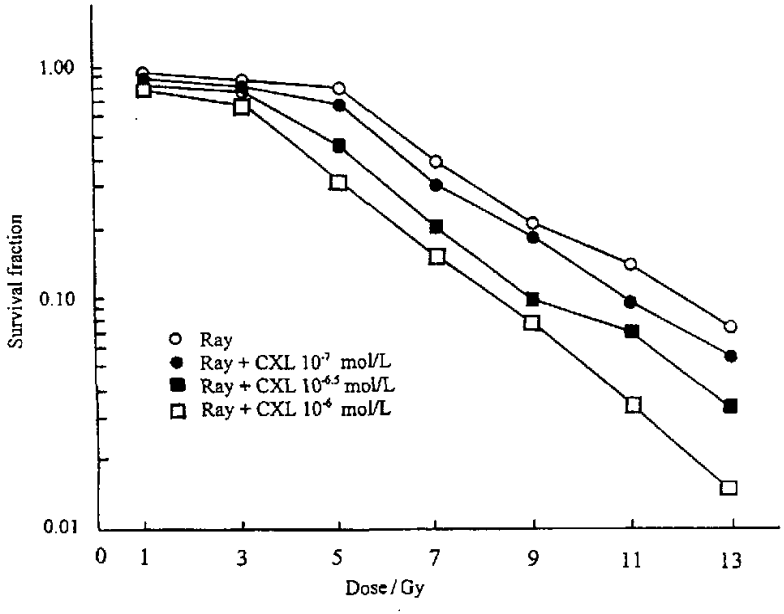

Fig.1 Effect of CXL on radiation dose-survival curve under hypoxic condition

Table 2 Increased radiosensitivity by CXL under hypoxic condition ($\bar{x} \pm s$ n=6)

CXL/mol· L^{-1}	SER		DDR/%
	Control D_0/test D_0	Control D_q/test D_q	
10^{-7}	1.07±0.25	1.16±0.28	10.07±2.30
$10^{-6.5}$	1.24±0.29	1.48±0.30	25.42±4.91
10^{-6}	1.43±0.23	1.72±0.36	35.69±6.26

3 Discussion

Irradiation is the first choice for the treatment of nasopharyngeal carcinoma. Radiation therapeutic technique on this kind of tumor has been continuously improved for recent decades, but the best 5-year survival rate stays in 30%~50% yet. Recurrent rates after irradiation is as high as 30%~40%[6]. Recurrence is related mainly with residual tumor following the first radiotherapy. These carcinoma cells are insensitive to ray, most of them are hypoxic cells. Enhancing sensitivity of hypoxic nasopharyngeal carcinoma cells to ray is, thereby, of great clinical significance.

The combination of irradiation with coix seeds in curing advanced nasopharyngesl carcinoma has obtained satisfactory short- and long-term results[7,8], which suggests that this Chinese herbal drug can increase the tumor radiosensitivity. The main active component of coix seed is CXL[1]. In hypoxic condition, low dose of CXL without cytotoxicity

produced a shift of radiation dose-survival curve of CNE-2Z to the left, with the reduction of D_0, D_q and N values. The decrease of D0 means the increase of susceptibility of CNE-2Z to radiation. The depression of D_q and N indicates the lowering of threshold dose for killing the cells by ray. Therefore, $10^{-7} \sim 10^{-6}$ mol/L of CXL made radiation dose dwindle 10.07%~35.69%, SER also increased with rise of CXL dose. The mechanism of enhancement by CXL probably has relation to its suppressing CNE-2Z sublethal damage repair because of the drop of Dq and N values. In this way, lower dose of ray caused CNE-2Z to be killed in exponential manner, which was characterized by the decrease of D_0 value. In addition, the results were similar regardless of whether CXL was administered before or after irradiation. Thus the radiation time might be ignored when coix seed or CXL is applied in clinic.

(The experiment on irradiation in this paper was performed in the affiliated tumor hospital of Sun yat-sen university of medical science.)

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