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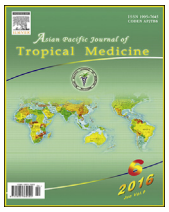

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Comparative study of the chitooligosaccharides effect on the proliferation inhibition and radiosensitization of three types of human gastric cancer cell line

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ABSTRACT

Objective: To observe the chitooligosaccharides (COS) effect on the proliferation inhibition and radiosensitivity of three types of human gastric cancer cell line.

Mothods: CCK-8 assay was employed to obtain the inhibition ratio of COS on BGC823 cells, MKN45 cells and SGC7901 cells at 48 h after treatment and the proliferation-inhibition curve was drawn with the inhibition ratio of COS on three types of cells. The clonogenic assay was used to detect the cell viability of 0, 1, 2, 4, 6 and 8 Gy (6 dose grades) in RAY group and RAY + COS group after X-ray, and the cell survival curve was used to analyze the sensitization enhancement ratio of COS. Flow cytometry was employed to detect cell cycle and apoptosis rate in control group, RAY group and RAY + COS group after 48 h treatment.

Results: COS inhibited the proliferation of three types of cells. The inhibition rate was positively correlated with the concentration of COS, and the susceptibility of MKN45 cells, SGC7901 cells and BGC823 cells to COS decreased in turn. The cell viability decreased gradually with the increasing radiation dose in RAY group and RAY + COS group ($P < 0.01$). The cell viabilities of RAY + COS group were lower than those of RAY group at all the dose grades under X-ray exposure ($P < 0.01$), and the sensitization enhancement ratios of COS on BGC823 cells, MKN45 cells and SGC7901 cells were 1.06, 1.28 and 1.15, respectively. In controlled trials, apoptosis rate and percentage in the G_2/M phase of three types of cells in RAY + COS group were higher than those in control group and RAY group, and percentage in the S phase and the G_0/G_1 phase in RAY + COS group were lower than those in the other two groups ($P < 0.01$).

Conclusions: COS can inhibit the proliferation of three types of human gastric cancer cells and enhance the radiosensitivity by inducing apoptosis and G_2/M phase arrest.

1. Introduction

With the progress of medical science and technology, the incidence and mortality of gastric cancer show a trend of decline gradually in the worldwide, but China is still a high-risk area where the cases of newly-increased and death in each year are more than 40% and 35% of total amount of the world [1]. A survey data of cancer epidemiology of China in 2010 showed that standardized incidence and mortality of gastric cancer all ranked third in malignant tumor, 23.71% and 16.64% respectively [2,3], and it's of great significance to actively explore the effective treatment for many patients with gastric cancer. Although surgical resection is still the preferred way to radically cure gastric cancer currently, the recurrence and mortality of a single surgery are more than 80% in 5 years after operation. Based on this background, radiotherapy as an irreplaceable and important supplementary method has been used in each stage of clinical treatment comprehensively [4,5]. Although radiotherapy shows more and more advantages in the treatment and research of gastric cancer, damage of radioactive rays on healthy tissue still can't be ignored. The use of excellent radiosensitizer is effective measure to lower the side-effect of radiotherapy. Chitooligosaccharides (COS) is one of the concerned hot-topics of biological medical workers in recent years and past research has found that it not only possesses the

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good effect like oxidation reaction and activation of autoimmune, but also has positive effect to inhibit tumor development [6,7]. However, the use of COS synergistic radiation is still seldom reported so far. This research selects three types of human gastric cancer cell line including BGC823, MKN45 and SGC7901 as objective to conduct an experiment to compare COS effect on their proliferation inhibition and radiosensitization. Moreover, we preliminary explore the mechanism of action and hope to provide theoretical basis for seeking more efficient and harmfulless treatment scheme for gastric cancer. Presently reports are the following.

2. Materials and methods

2.1. Cell lines, experimental materials and experimental methods

A total of 6 concentration levels of COS were established and each level was conducted simultaneously with 3 holes in parallel samples. Three cell lines of continuous cell culture to logarithmic phase (human gastric cancer including BGC823, MKN45, SGC7901 cells, Shanghai Institute of Cells, Chinese Academy of Sciences) were diluted as 4×10^4/L of concentration through digestion and joined into 96-well plates according to 0.1 mL/well, and then were placed in a suitable environment for adherent growth (CO_2 incubator, Shanghai Gemtop Scientific Instrument Co., LTD). The fresh culture medium (0.11 mL/well) diluted with COS (Shanghai HuiCheng Biotechnology Co., LTD) was replaced after 24 h and 0, 1.0, 2.0, 3.0, 4.0, 5.0 mg/mL of concentrations respectively were added into COS in each well. After infiltrating 48 h, CCK-8 reagent was added into COS along the well (0.01 mL/well) (CCK-8 kit, Shanghai LiRui Biological Technology co., LTD), and then the reagent and culture solution were mixed by tapping culture plate. All levels of absorbance (OD) were detected at $\lambda = 450$ nm in 4 h after reacting fully. The experiment was repeated for 3 times to investigate the proliferation suppression effect of COS on three types of cells, which is the basis to select 1.0 mg/mL of COS concentration to conduct a follow-up study.

A total of 6 dose levels of X-ray were established, and each level was divided into RAY group and RAY + COS group and each group was conducted simultaneously with 3 holes in parallel samples. Different concentrations of single-cell suspension were joined into 6 wells culture plate according to the inoculation amount of 0 Gy dose level (200/well), 1, 2 Gy dose level (500/well), 4, 6 Gy dose level (2000/well) and 8 Gy dose level (5000/well), and were placed in a suitable environment for adherent growth. Appropriate COS (1.0 mg/mL) was added into each well of RAY + COS group after 6 h and equivalent quantity of culture medium was added into each well of RAY group after infiltrating for 48 h. Tissue analog with about 1 cm thickness was affixed on the culture plates in two groups and was conducted with X-ray irradiation in a distance of 100 cm and dosage rate of 2 Gy/min. (electron linear accelerator, Nanjing Chuang Rui Ying Biological Technology Co., LTD). Culturing for 12 d continuously, the number of cell mass consisted over 50 U was calculated through washing, fixing and dyeing. The experiment was repeated for 3 times to calculate data and draw up the cell survival curve, and sensitization enhancement ratio was calculated by the value of final slope (D_0): $SER = D_{0(RAY)}/D_{0(RAY + COS)}$.

Three groups were established and conducted simultaneously with 3 holes in parallel samples. The cell suspension of 1×10^5/L concentration was inoculated into 6-well plates and was placed in a suitable environment for adherent growth for 24 h. Appropriate COS (1.0 mg/mL) was added into RAY + COS group and equivalent quantity of culture medium was added into non-treatment group and RAY group. RAY group and RAY + COS group were infiltrated for 48 h, and then received 6 Gy X-ray irradiation. Cultivating 48 h after replacing fresh medium, cell cycle and apoptosis rate in all groups were detected through the processing of digestion, wash and dilution.

2.2. Statistical methods

SPSS19.0 statistical software was used to perform statistical analysis. The distribution ratio of OD value, survival rate, apoptosis rate and cell cycle were all expressed as mean ± SD. ANOVA was used to analyze the OD value of various COS concentration levels and cell survival rate of X-ray dose levels among many groups. *T*-test was used to compare the cell survival rate under all X-ray doses of RAY group and RAY + COS group, and cell cycle and apoptosis rate in the third group of control group. $P < 0.05$ showed statistical significance.

3. Results

3.1. Anti-proliferative effect of COS on BGC823 cells, MKN45 cells and SGC7901 cells

OD values of BGC823 cells, MKN45 cells and SGC7901 cells under COS concentrations (0 mg/mL) were used as reference standard. The cell survival rate of three types of cells was decreased with a higher concentration of infiltrating after disposing various COS concentrations for 48 h and the difference was statistically significant ($P < 0.01$). The COS was

Table 1
Anti-proliferative effect of COS on three types of cells.

COS concentration (mg/mL)	BGC823		MKN45		SGC7901		*P*	*F*
	OD	Inhibition rate (%)	OD	Inhibition rate (%)	OD	Inhibition rate (%)		
0	0.983 ± 0.012	0.00	0.993 ± 0.010	0.00	0.984 ± 0.008	0.00	0.074	2.09
1.0	0.929 ± 0.019	5.49	0.895 ± 0.013	8.95	0.910 ± 0.016	7.52	0.040	6.95
2.0	0.851 ± 0.015	13.43	0.797 ± 0.031	18.92	0.820 ± 0.023	16.67	0.003	60.7
3.0	0.755 ± 0.023	23.19	0.687 ± 0.025	30.82	0.715 ± 0.017	27.34	0.000	156.2
4.0	0.645 ± 0.011	34.38	0.566 ± 0.015	43.00	0.596 ± 0.018	39.43	0.000	198.5
5.0	0.521 ± 0.019	47.00	0.425 ± 0.024	57.20	0.478 ± 0.024	51.42	0.000	263.4
F	304.6	–	352.8	–	337.1	–	–	–
P_{ANOVA}	0.000	–	0.000	–	0.000	–	–	–