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ORIGINAL ARTICLE

Proliferation inhibition and apoptosis of breast cancer MCF-7 cells under the influence of colchicine

Yongjie Sun[1], Xiaoyan Lin[2], Hong Chang[3]

[1]Department of Breast Thyroid Surgery, Shandong Province Hospital affiliated to Shandong University, Jinan, Shandong, 250021, China; [2]Department of Pathology, Shandong Province Hospital affiliated to Shandong University, Jinan, Shandong, 250021, China; [3]Department of Hepatobiliary Surgery, Shandong Province Hospital, affiliated to Shandong University, Jinan, Shandong, 250021, China

Summary

Purpose: *This study was designed to explore the effect of colchicine on the proliferation and apoptosis of Breast Cancer Michigan Cancer Foundation – 7 (MCF-7) cells.*

Methods: *The experiment was conducted at the University Laboratory of East Provincial Hospital in April, 2015. The first inhibitory effect of colchicine on breast cancer MCF-7 cells was observed by MTT (3-(4,5)-dimethylthiahiazo(-z-y1)-3,5-diphenytetrazoliumromide) assay, and then the effect of colchicine on apoptosis of breast cancer MCF-7 cells was measured by flow cytometry.*

Results: *The colchicine's inhibitory effect on breast cancer MCF-7 cells gradually increased with increasing concentration and longer exposure time. Breast cancer MCF-7 cells showed different levels of apoptosis with different colchicine concentrations at 24th, 48th and 72nd hrs, and the apoptosis rate tended to be higher with increasing concentration and prolonged exposure time.*

Conclusion: *All the findings suggest that colchicine is able to inhibit proliferation of breast cancer MCF-7 cells and induce cell apoptosis, and the intensity of the effect was associated with dosage and time.*

Key words: *breast cancer, cell apoptosis, colchicines, MCF-7 cells, proliferation inhibition*

Introduction

Breast cancer, a frequently seen malignant tumor, is mostly treated by combined treatment modalities [1-3] including surgery, chemotherapy [4], radiotherapy, hormonotherapy and molecular targeting treatment. Though drugs for treating breast cancer are diverse, cancer cells become resistant in clinical practice [5]. What is worse, many drugs are accompanied with severe side effects. As a result, searching for a drug with good therapeutic effect, less side effects and effective inhibitory effect on the occurrence and development of breast cancer has become one of the most important topics [6].

Treating breast cancer with traditional Chinese medicine lasts for a long time in China and thus a special theoretical system has been formed. Traditional Chinese medicine is supposed to be effective in prolonging lifetime, improving the quality of life, reducing side effects produced by chemoradiotherapy, as well as enhancing treatment efficacy [7]. It has been reported that medicinal herbs like berberine, liquiritigenin and hydroxycamptothecin can inhibit the proliferation of breast cancer MCF-7 cells, as well as induce cell apoptosis [8-10]. Edible tulip bulb with acrid and sweet flavor has favorable effects in treating advanced breast cancer, and it is frequently used for relieving toxicity in breast cancer treatment. Colchicine, the main component of edible tulip bulb, is a natural tubulin inhibitor with sound an-

Correspondence to: Hong Chang, MD. 324, Jing Wu Road, Shandong Province Hospital, Jinan, 250021, China. Tel: +0531-68773232, E-mail : ch150609@163.com
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titumor activity [11].

This study was carried out to assess in vitro the effects of colchicine on the proliferation and apoptosis of breast cancer MCF-cells, and to analyze its mechanism of action. This work aimed to provide a theoretical and experimental basis for preparing high-efficient and multiple-target drugs used for relieving toxicity and destroying tumor clinically.

Methods

The aim of the study was to observe the inhibitory effect of colchicine on the proliferation of breast cancer MCF-7 cells with MTT (3-(4,5) - dimethylthiahiazo (- z-y1) - 3, 5-diphenytetrazoliumromide) assay.

Observation of the inhibitory efect of colchicine on the proliferation of MCF-7 cells using MTT

Grouping

Colchicine (Keygen Biotech, Nanjing, China) was dissolved with 0.2% dimethylsulfoxide (DMSO) (Art. No. 20120322, Jiuyi Reagent Company, Shanghai, China) to get concentrations of 0.3125, 0.625, 1.25, 2.5, 5, 10, 20 and 40 μg/ml (colchicine group). A negative control group (without colchicine) and a positive control group (with colchicine 10 μg/ml) were created. Except for drug use, the other conditions of the two control groups were the same with the colchicine group. Cells were incubated for 24, 48 and 72 hrs.

Experimental method

Breast cancer MCF-7 cells (Keygen Biotech, Nanjing, China) that were in the logarithmic phase of growth were first prepared into 5×10^4/ml cell suspension after digestion. Then, the cell suspension was added into 96-well plate, with 100 μl (5×10^3/cells) in each well. After that, the 96-well plate was incubated in an incubator (XD-101, SANYO, Japan) containing 5% CO_2 at 37 °C for 24 hrs. After removal of the medium prepared using RPMI-1640, the cells were washed twice by phosphate buffered solution (PBS) (Art. No. KGB500, Keygen Biotech, Nanjing, China). Then, colchicine was dissolved with complete medium and added with medium containing colchicine 200 μl in each well. Positive control group and negative control group were also set up. Afterwards, the 96-well plate was incubated in the incubator containing 5% CO_2 and at 37 °C for 24, 48 and 72 hrs. When the culture was finished, optical density (OD) was measured on cells stained by MTT (λ=490 nm). Detailed procedures were as follows: Firstly, each well was added with 20 μL MTT (5 mg/ml) and then they were incubated for 4 hrs; after culture medium removal each well was added with 150 μL DMSO, and then the plate was shaken up for 10 min; next, OD value was measured by a microplate reader (Elx800, BioTek, USA) (λ=490 nm), and finally the inhibition rate and IC_{50} of each group was calculated. The formula for calculating the inhibition rate was:

$$Inhibition\ rate\ (\%) = \frac{OD\ value\ of\ neg\ control\ group - OD\ value\ of\ exp\ group}{OD\ value\ of\ neg\ control\ group} x100\%$$

IC_{50} was calculated by Bliss method using SPSS 17.0 software.

Detection of the effect of colchicine on apoptosis and proliferation of breast cancer MCF-7 cells with flow cytometry

Grouping

Blank control group (without drugs), low-concentration colchicine group (2.5 μg/ml), medium-concentration colchicine group (10 μg/ml) and high-concentration colchicine group (40 μg/ml) were set up. The treatment times were 24, 48 and 72 hrs, respectively.

Experimental method

Cells that were in the logarithmic phase of growth were inoculated into 6-well plate after digestion. When cells were adhered next day, the corresponding medium containing different concentrations of colchicine was added into different groups, and, meanwhile, the negative control group was set up. At 24th, 48th and 72nd hrs, cells were digested by 0.25% pancreatin (without ethylene diamine tetraacetic acid / EDTA) and washed by PBS twice, followed by centrifugation at 2000 rpm for 5 min. Finally 5×10^5 cells were collected. Then, 500 μL binding buffer was added to suspend cells, followed by 5 μL Annexin V-FIT and 5 Annexin V-FIT propidium iodide. Then, the cells were maintained at room temperature in the dark for 5-10 min. Flow cytometry (FACS Calibur, Becton-Dickinson, USA) was used to measure the apoptosis of cells.

MCF-7 cells in the logarithmic phase of growth were inoculated into 6-well plate. When cells were adhered next day, the corresponding medium containing different concentrations of colchicine was added. Meanwhile, the negative control group was set up. At 24th, 48th and 72nd hrs, cells were digested by 0.25% pancreatin (without EDTA); then the collected cells were washed by PBS once, followed by centrifugation at 2000 rpm for 5 min. Finally 5×10^5 cells were collected. The prepared single-cell suspension was fixed by ethyl alcohol with 70% volume fraction for 2 hrs or overnight and then preserved at 4 °C. Stationary liquid were removed by PBS before staining. Cell suspension could be screened once by 200-mesh sieve if necessary. Water bath (37 °C) was used after 100 nL RNaseA was added. Cells stained by 400 nL propidium iodide were kept in the dark at 4 °C. Thirty min later, red fluorescence was measured with optical maser at 488 nm wavelength.

Table 1. MTT assay results obtained from breast cancer MCF-7 cells treated with different concentrations of colchicine for 24, 48 and 72 hrs

Group (μg/ml)	24 hrs		48 hrs		72 hrs	
	OD value (mean ± SD)	Inhibition rate (%)	OD value (mean ± SD)	Inhibition rate (%)	OD value (mean ± SD)	Inhibition rate (%)
Colchicine	0.319±0.319**	21.9	0.351±0.006**	44.1	0.222±0.002**	75.8
0.3125	0.393±0.028	3.68	0.586±0.009**	7.28	0.720±0.012**	21.43
0.625	0.385±0.006	5.72	0.572±0.003**	9.49	0.709±0.007**	22.6
1.25	0.370±0.012**	9.13	0.546±0.011**	13.61	0.672±0.007**	26.6
2.5	0.359±0.008**	12.01	0.515±0.008**	18.46	0.651±0.008**	28.89
5	0.351±0.014*	13.89	0.510±0.004**	19.3	0.602±0.005**	34.28
10	0.344±0.005**	15.77	0.484±0.005**	23.42	0.500±0.009**	45.38
20	0.342±0.007**	16.09	0.465±0.007**	26.37	0.392±0.004**	57.24
40	0.332±0.005**	18.71	0.438±0.007**	30.75	0.335±0.011**	64.43

OD: optical density, SD: standard deviation, *p<0.05, **p<0.01 compared with the control group

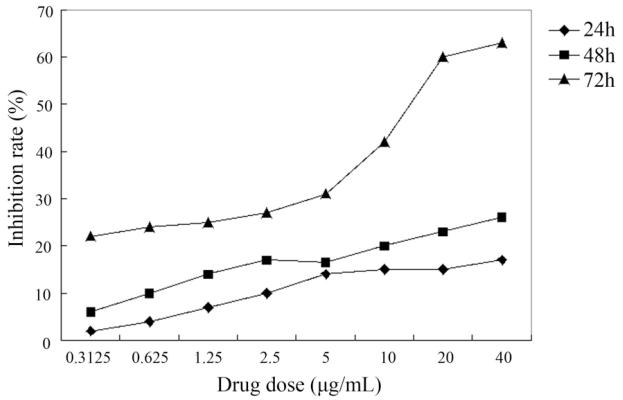

Figure 1. Inhibitory effect of colchicine of different concentrations on the proliferation of breast cancer MCF-7 cells.

Statistics

All experimental data were expressed as mean±SD. Excel 2003 and SPSS 17.0 were used for data processing and analysis. Correlation analysis was performed by homogeneity test of variance as well as one way analysis of variance (ANOVA). A p value <0.05 showed statistical significance.

Results

Effect of colchicine on the proliferation of breast cancer MCF-7 cells

The inhibitory effects of colchicine with different concentrations on the proliferation of breast cancer MCF-7 cells are shown in Table 1 and Figure 1. It was found that the inhibitory effect on breast cancer MCF-7 cells gradually increased with increasing concentration of colchicine and increasing time of exposure. Compared to negative control group, groups except those with 0.3125 μg/ml and 0.625 μg/ml colchicine (acting for 24 hrs) showed statistical significance (p<0.01). This revealed that colchicine possessed inhibitory activity on the proliferation of breast cancer MCF-7 cells, showing obvious relationship between dose-effect and time-effect.

Table 2. Effects of colchicine on the apoptosis of breast cancer MCF-7 cells

Treatment time (hours)	Group	Total apoptosis rate (%)
24	Negative control group	6.6
24	Colchicine 2.5 μg/ml	13.1
24	Colchicine 10 μg/ml	16.4
24	Colchicine 40 μg/ml	21.5
48	Negative control group	7.3
48	Colchicine 2.5 ug/ml	18.1
48	Colchicine 10 μg/ml	25.8
48	Colchicine 40 μg/ml	42.4
72	Negative control group	8.1
72	Colchicine 2.5 μg/ml	30.9
72	Colchicine 10 μg/ml	43.8
72	Colchicine 40 μg/ml	65.9

Effect of colchicine on apoptosis of breast cancer MCF-7 cells

Apoptosis of breast cancer MCF-7 cells treated with colchicine with different concentrations for different times are shown in Table 2. All groups showed different levels of apoptosis of MCF-7 cells. Overall, the apoptosis rate tended to be higher with increased concentration and time of exposure. Colchicine showed the strongest abil-

Table 3. Effects of colchicine on the proliferation of breast cancer MCF-7 cells

Time (hours)	Group	G1 (%)	S (%)	G2 (%)	Apoptosis rate (%)
24	Negative control group	55.4	27.98	16.62	0
24	Colchicine 2.5 μg/ml	53.83	28.99	17.18	0
24	Colchicine 10 μg/ml	50.84	28.14	21.02	2.34
24	Colchicine 40 μg/ml	48.25	27.33	24.42	4.57
48	Negative control group	52.15	29.7	18.15	0
48	Colchicine 2.5 μg/ml	47.14	29.46	23.4	4.64
48	Colchicine 10 μg/ml	45.72	28.84	25.44	6.76
48	Colchicine 40 μg/ml	43.74	27.62	28.64	6.59
72	Negative control group	54.58	27.89	17.53	0
72	Colchicine 2.5 μg/ml	45.25	27.68	27.07	4.87
72	Colchicine 10 μg/ml	42.71	26.02	31.27	5.58
72	Colchicine 40 μg/ml	34.21	28.77	37.02	18.97

ity of inducing apoptosis when MCF-7 cells were treated with 40 μg/ml for 72 hrs, and the total apoptosis rate at 72 hrs was 65.9%.

Effect of colchicine on the proliferation of breast cancer MCF-7 cells

The effect of colchicine in low, medium and high concentration on breast cancer MCF-7 cells at 24th, 48th and 72nd hrs is shown in Table 3. Compared to the negative control group, the proportion of cells in G0/G1 phase gradually decreased as the concentration and the time of exposure increased; cells in the S phase remained the same and cells in the G2/M phase tended to be increased with increasing concentration and time of exposure. Moreover, the cell cycle remained at G2/M phase and cells in G2/M phase accumulated, showing a close correlation with time. The apoptosis rate of MCF-7 cells reached the highest value (18.97%) when the cells were treated with 40 μg/ml colchicine for 72 hrs.

Discussion

Breast cancer, is the most common malignancy in females, with increasing tendency, accounting for 7-10% of malignant tumors occurring across the whole body, which severely threatens the physical and psychological health of the patients [12]. Multidisciplinary treatments gradually take the place of single surgical treatment used previously due to the rapid development of modern medical science and tumor treatment concept. Western medicine is inclined to treat breast cancer with surgical operation, radiotherapy, cytotoxic chemotherapy, hormonotherapy, molecular targeting treatment and biotherapy.

Colchicine, as an alkaloid, has attracted the attention of scientists for its special structure and strong antitumor activity since its discovery. It shows a favorable therapeutic effect on breast cancer, liver cancer, esophageal cancer, lung cancer and gastric carcinoma [13] and it has been reported that can reduce the lesions of patients with breast cancer. In combination with radiation therapy colchicine can improve the long-term survival in cervical cancer and demecolcine ointment can improve 3-year non-recurrence rate to 83% if used in non-metastatic skin cancer [14]. Tian et al. [15] found that colchicine could inhibit the growth of C6 glioma cells in rats and the highest inhibition rate could exceed 80% as assessed in vitro with MTT assay.

Colchicine is a natural-derived tubulin inhibitor and also a strong inhibitor of cell mitosis. It restrains cell mitosis through sealing the site where tubulin dimers form, interfering in normal microtubule functions and inhibiting the formation of the spindle.

The most obvious feature distinguishing cancer cells from normal cells is their rapid proliferation [16]. Thus inhibiting cancer cells' proliferation is one of the antitumor mechanisms.

MTT assay used in this study revealed that colchicine showed inhibitory activity in the proliferation of MCF-7 cells, and this activity became strongest (57.24-64.43%) when the concentration of colchicine was 20μg/ml or higher and the treatment time was 72 hrs, but the inhibition rate was below 50% in the negative control groups. Moreover, the inhibitory effect was supposed to be associated with time and dosage.

Cell apoptosis, also called programmed cell death, is a gene-regulated cell death actively occurring in specific time periods [17]. Traditional Chinese medicine is supposed to be able to interfere in the growth and proliferation of tumor cells through multiple mechanisms and finally inducing programmed cell death. Jo et al. [18] found that liquorice could inhibit the proliferation of MCF-7 cells showing a time- and dose-dependent relationship; liquorice extract was able to induce DNA breakage; moreover, the inhibition mechanism of liquorice on MCF-7 cells might be associated with blockage of cells in the early G1 phase. Through analysis, the authors finally found that liquorice inhibited the growth of cells and induced their apoptosis by regulating the expression of apoptosis-related Bcl-2/Bax protein. In addition, Liu et al. [19] concluded from their experiment that Cordyceps sinensis could induce apoptosis of breast cancer cells, proving a theoretical basis for its antitumor activity. They also found that apoptosis was induced by regulating the expression of Bcl-2/Bax gene.

This study detected the activity of colchicine on breast cancer MCF-7 cells with flow cytometry. It was observed that MCF-7 cells showed different levels of apoptosis after being treated with colchicine in different concentrations for 24, 48 and 72 hrs and that apoptosis rate increased as concentration and treatment time increased; colchicine's ability in inducing cell apoptosis was strongest when the concentration was 40 μg/ml and the exposure time was 72 hrs, with the overall apoptosis rate at that time being 65.9%. Finally, it was found that colchicine could block cell cycle, inhibit DNA synthesis of MCF-7 cells and induce apoptosis, this effect being associated with exposure time and dosage.

Anticancer agents can be divided into cell cycle-specific and non-specific, the former exerting functions in a certain phase of the cell cycle. To date, it has been found that some traditional Chinese drugs with antitumor activity can be used in a certain phase of the cell cycle to block it and induce apoptosis [20].

The present work detected the effects of colchicine on MCF-7 cells with different concentrations and treatment times. Compared with the negative control group, the proportion of cells in G0/G1 phase gradually decreased, and cells in G2/M increased with higher agent's concentration and prolonged exposure time, leading to cell cycle blockage in G2/M phase with cells accumulated in this phase. This effect indicated an obvious correlation between time and effect and suggested that the inhibitory effect and apoptosis-inducing capacity of colchicine to breast cancer cells was associated with mitosis disturbance which is caused by cells blocked in G2/M phase.

To sum up, we explored the effect of colchicine on the proliferation and apoptosis of MCF-7 with MTT assay and flow cytometry. Colchicine was proved to be able to inhibit the proliferation of these cells, induce cell apoptosis, and the effect was dose- and time-dependent. Its mechanism of action is supposed to be associated with blockage of cells in the G2/M phase.

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Conflict of interests

The authors declare no confict of interests.

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