

STUDY ON THE INHIBITORY EFFECT OF ALLICIN ON HUMAN GASTRIC CANCER CELL LINE SGC-7901 AND ITS MECHANISM

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* E-Mail: onetgifnd@126.com**Abstract**

Background: Allicin is the main active constituent of *Allium sativum* L., which is characterized by broad antibacterial spectrum (MarkosN et al., 2008; Chen et al., 2008); it also has apparent inhibitory effects on a variety of tumors. The Objective of the paper is to study the inhibitory effect of allicin on human gastric cancer cell line SGC-7901.

Materials and Methods: MTT assay and flow cytometry technique were applied to determine the inhibition rate of allicin on human gastric cancer cell line SGC-7901. The results shows that different concentrations of allicin apparently inhibited the gastric cancer SGC7901 cells, cell growth inhibition rates in the experimental groups showed an upward trend with increased allicin concentration, which were concentration-dependent.

Results: Flow cytometry results found that the cell cycle was arrested in the G2/M phase. Allicin has an apparent inhibitory effect on proliferation of gastric cancer cells, and can induce their apoptosis.

Conclusion: Compared with other chemotherapeutic drugs, allicin's anti-tumor effect is better; and toxic and side effects are relatively small.

Keywords: Allicin; human gastric cancer cell line SGC-7901; flow cytometry.

Introduction

Allium sativum L. is the bulbs of garlic in the genus *Allium*, family Liliaceae in the clade Monocots, which is widely cultivated in China. *Allium sativum* L. is rich in nutrient, each 100 g of fresh *Allium sativum* L. contains 414 g of protein, 23 g of carbohydrate, 5 mg of calcium, 0.2 g of fat, 0.4 mg of iron, 0.24 mg of thiamine, 0.03 mg of riboflavin, 0.9 mg of niacin, 3 mg of vitamin C, 0.7 g of crude fiber, 0.2 g of garlic oil, as well as over 30 kinds of volatile sulfur compounds (Sun et al., 2009). Allicin is the main active constituent of *Allium sativum* L., which is characterized by broad antibacterial spectrum (Markos et al., 2008; Chen et al., 2008); it also has apparent inhibitory effects on a variety of tumors such as liver cancer, stomach cancer, colon cancer and lung cancer. The mechanisms of its anti-tumor action mainly include exertion of antioxidant effect by scavenging reactive oxygen radicals, induction of apoptosis, and inhibition of the proliferation of human tumor cells (Hassan, 2004; Arranz et al., 2007; Tian et al., 2006). In this experiment, allicin was acted on human gastric cancer cell line SGC-7901 to study the tumor cell killing effect of allicin.

Materials and methods

Human gastric carcinoma metastatic lymph node cell line SGC-7901, (Tumor Research Institute, The First Hospital of China Medical University), Allicin injection (Jiangsu Chia-tai Tianqing Pharmaceutical Co., Ltd. batch number: 366-589-12), MTT (Sigma), RPMI-1640 (Gibco, USA); flow cytometer (FACScan, B.D USA).

Cell cultivation

Human gastric cancer SGC-7901 cells were cultured in RPMI 1640 medium (containing 10% fetal bovine serum), digested and passaged with 0.25% trypsin, and cultured routinely in the incubator (conditions: 37°C, 5% CO₂), cells in the logarithmic growth phase were selected for the experiment.

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Grouping

The cells were divided into five groups, namely the blank group, control group (containing only cultured cells without drug intervention), and allicin groups (3, 6, 12 mg/L).

Determine of cell growth inhibition rate by MTT assay

Gastric cancer SGC-7901, cells in the logarithmic growth phase were collected, digested conventionally with trypsin and made into single cell suspension, cell density was adjusted to 5×10^7 cells/L, the cells were then seeded in 96-well plates at 0.1 ml per well, 24 hrs after cultivation (conditions: 37°C, 5% CO₂), different concentrations of allicin solutions were added, and the cultivation was continued for another 48 hrs, then culture medium was discarded, and serum-free culture medium and MTT were added at 100 µL/well and 20 µL/well, respectively, after four additional hours of cultivation, 0.1 ml of dimethyl sulfoxide (DMSO) was added and the plates were shaken for 10 min, then absorption was measured at 570 nm wavelength. Cell growth inhibition rate = (A control group - A drug group) / A control group × 100%. Inhibition curve was plotted, and the half maximal inhibitory concentration (IC₅₀) of the drug was calculated.

Flow cytometry detection of apoptosis rate

Gastric cancer SGC7901, cells were collected at 12, 24 and 48 hrs after the action of allicin (3 mg/L), on them, the harvested cells in each group were made into single cell suspension, and fixed for 24 hrs by slowly adding precooled 70% ethanol, then washed twice with PBS, equal volumes of cell suspension and PI dye were mixed, and allowed to stand at 4°C for 30 min, samples were placed into the sample chamber of flow cytometry (FCM), and apoptosis rate of gastric cancer SGC7901 cells was detected on flow cytometry. The experiment was repeated five times for each group.

Flow cytometry detection of effect of allicin on cell cycle

Gastric cancer SGC7901, cells were collected and seeded in culture flasks at 5×10^4 ml/L, after culturing for 48 h, different concentrations of allicin were added, after culturing for another 12, 24 hrs, respectively, culture medium was discarded, followed by digestion, centrifugation for 8 min., to collect cells. The cells were washed 3, times with PBS, stained at 4°C for 30 min., by the addition of 10 µg/ml RNase-containing PI. After filtration, cell cycle was detected on flow cytometry. The experimental results for percentages of each phase of cell cycle and hypodiploidy were analyzed using cellQuest software. The experiment was repeated three times.

Statistical processing

The experimental results were analyzed using SPSS 13.0 software, comparison among groups was performed by analysis of variance, the results were expressed $\bar{x} \pm s$, a significance level of $\alpha = 0.05$ was applied.

Results

Inhibitory effect of allicin on gastric cancer SGC7901 cells

The cells in the control group presented normal logarithmic growth, different concentrations of allicin apparently inhibited gastric cancer SGC7901, cells, cell growth inhibition rates in the experimental groups showed an upward trend with increased allicin concentration, which were concentration-dependence.

Apoptosis inducing effect of allicin on gastric cancer SGC7901 cells

As can be seen from Fig. 2, compared with the blank control group, 12, 24 and 48 hrs, after the action of different concentrations of allicin

on gastric cancer SGC7901 cells, the apoptotic rates were apparently elevated, and the differences were significant.

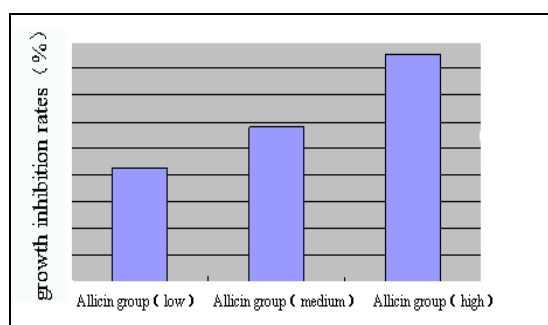


Figure 1: Inhibitory effect of allicin on SGC7901 cell proliferation

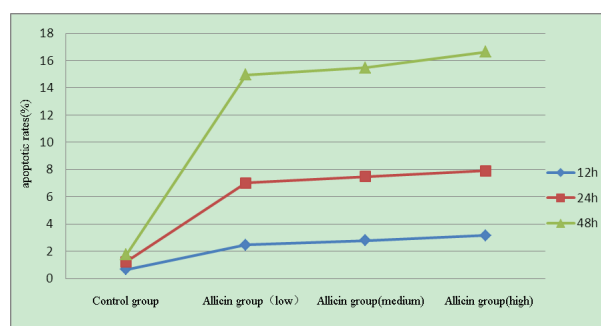


Figure 2: Antileptic effect of allicin on SGC7901 cell apoptosis

Effect of allicin on cell cycle

Flow cytometry results showed that 24 and 48 hrs, after the action of different concentrations of allicin, cell cycle of SGC7901, cells changed. Number of G₀/G₁ phase cells decreased slightly, G₂/M phase cells increased apparently, while S phase cells changed little. See Tab. 1, Table 2.

Table 1: Effect of allicin on cell cycle of SGC7901 cells (%)

Group	Dose (mg/L)	Action time (h)	G ₀ /G ₁ phase	S phase	G ₂ /M phase
Control group			62.13±2.13	13.54±1.47	24.33±2.69
Allicin group	3	24	59.23±2.15*	16.21±1.67*	24.56±2.53*
	6		58.76±2.34*	15.75±1.79*	25.49±2.37*
	12		58.32±2.27*	15.23±1.52*	26.45±2.79*

Note: Comparison with the control group, * P<0.01

Table 2: Effect of allicin on cell cycle of SGC7901 cells (%)

Group	Dose (mg/L)	Action time (h)	G ₀ /G ₁ phase	S phase	G ₂ /M phase
Control group			--	--	--
Allicin group	3	48	58.31±2.72*	15.83±1.45*	25.86±2.77*
	6		58.35±2.53*	14.55±1.49*	27.14±2.41*
	12		57.52±2.31*	13.49±1.63*	28.99±2.56*

Note: Comparison with the control group, * P<0.01

Discussion

Cell cycle refers to the whole complete cycle of cells from the end of first division to the end of next division, which is generally divided

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into four phases, namely G₁ phase, the pre-DNA synthesis phase; S phase, the DNA synthesis phase; G₂ phase, the post-DNA synthesis phase and M phase, the mitotic phase. While G₀ phase is the cell resting phase. The key point of cell proliferation lies in the G₁ phase, which can prevent DNA damaged cells from entering into the replication in S phase, it is the driving mechanism of cell cycle, which determines whether the cells can pass through the G₁ phase into S phase. G₂/M phase is the monitoring mechanism of cell cycle, which can ensure complete and accurate replication of cells; G₂ phase detection points can prevent damaged and unreplicated DNAs from entering into mitosis (Nasmyth K, 1996; Zeng, 1999; Heerdt et al., 2000).

In this experiment, after using different concentrations of allicin to act on human gastric cancer SGC7901 cells, MTT assay and flow cytometry technique were applied to study in-depth the effects of allicin on tumor cell inhibition and tumor cell cycle. The determination results showed that the growth of tumor cells was apparently inhibited in a concentration-dependent manner. After action of 3 mg/L allicin on human gastric cancer SGC7901 cells, apoptosis was induced, the apoptosis rates at 12, 24 and 48 h were 2.4%, 6.8% and 17.1%, respectively, which were all significantly higher than the control group.

The flow cytometry results found that the cell cycle was arrested in G₂/M phase. Different concentrations of allicin can arrest the damaged cells in the G₂/M phase of cell cycle, thus increasing the repair of damaged DNA, reducing chromosomal aberrations, and thereby selectively acts on G₂/M phase of gastric cancer cells and participates in cell cycle regulation (Sakamoto et al., 1997; Cao et al., 1996; Yu et al., 2001).

In conclusion, allicin has a significant inhibitory effect on proliferation of gastric cancer cells, and can induce their apoptosis. Compared with other chemotherapeutic drugs, its anti-tumor effect is better; toxic and side effects are relatively small. The in-depth study of the molecular mechanisms of its action is of great significance for expanding the clinical application and product development of allicin.

References

1. Arranz, N., Haza, A. I., and Garca, A. (2007) Protective effects of organosulfur compounds towards N-nitrosamine-induced DNA damage in the single-cell gel electrophoresis (SCGE) /HepG2 assay. *Food and Chemical Toxicology*, **45**: 1662-1669
2. Cao, H., Yang, H., Wu, W., and Zheng, S. (1996) Study of the effect of allicin on tumor cell cycle by flow cytometry. *Chinese Journal of Cancer*, **15**(6): 401-404
3. Chen, X. Y., Zhao, C. H., Liu, S., and Liang, J. H. (2008) Researches of Antibacterial Activities of Allicin in Vitro. *Journal of Shenyang Agricultural University*, **39**(1): 108-110
4. Hassan, H. (2004) Ajoene (naturalgarlic compound): a new anti-leukaemia agent for AML therapy. *Leuk Res*, **28**(7): 667-671
5. Heerdt, B. G., Houston, M. A., Mariadason, J. M., and Augenlicht, L. H. (2000) Dissociation of staurosporine induced apoptosis from G₂-Marrest in SW620 human colonic carcinoma cells: initiation of the apoptotic cascade is associated with elevation of the mitochondrial membrane potential (deltapsim). *Cancer Res*, **60**: 6704-6713
6. Markos, N. M., Athanasios, S., and Haralambos, B. (2008) In vitro bactericidal activity of allicin combined with cefoperazone, tobramycin and ciprofloxacin. *International Journal of Anti microbial Agents*, **31**: 175-187
7. Nasmyth, K. (1996) View point: putting the cell cycle in order. *Science* **274**: 1643-1645
8. Sakamoto, K., Lawson, L. D., and Milner, J. A. (1997) Allyl sulfides from garlic suppress the in vitro proliferation of human A549 lung tumor cells. *Nutr Cancer*, **29**(2): 152-156
9. Sun, C. L., and Yu, D. S. (2009). The Extraction and Utilization of Allicin [J]. *Guangzhou Chemical Industry*, **37**(6): 65-67
10. Tian, X. J., and Kang, J. (2006) The Studies of Allicin on Induction of the Apoptosis in Human Laryngeal Squamous Cell Carcinoma Strain Hep-2. *Journal of Jinzhou Medical College*, **27**(6): 21-24
11. Yu, C. Q., Cai, Z. L., Yan, Y. B., Zhang, Y. N., Pan, R. P., and Ling, C. Q. (2001) Inhibitory effect of allicin on growth of human ovary cancer cell line HO8910. *Journal of Anhui Traditional Chinese Medical College*, **20**(6): 34-36
12. Zeng, Y. X. (1999) *Oncology*. 1st edition, Beijing: People's Medical Publishing House, 107-112