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Original Article

Effect of selenium on cancer cells of S180 sarcoma-bearing mice

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Abstract: Selenium participates in several tumors. Selenium has certain preventive and therapeutic effects on tumors. In this study, different concentrations of selenium were used to treat a sarcoma mouse model, and the inhibition rate of tumors and the apoptosis rate of cancer cells were observed. S180 sarcoma cell line was inoculated into nude Kunming mice. Each group was injected intraperitoneally with different concentrations of sodium selenite. The mice were sacrificed on the 10th and 20th day after inoculation followed by analysis of the tumor inhibition rate and cell apoptosis by flow cytometry. The tumor weight in the experimental group was significantly reduced compared with control group. With increased concentrations of sodium selenite, tumor weight was gradually decreased ($P<0.05$) and tumor inhibition was enhanced gradually ($P<0.05$). Cell apoptosis rate in the experimental group was significantly increased compared to control group. With increased concentration of sodium selenite, cell apoptosis was gradually increased and was slightly higher on the 20th day than that on the 10th day. Selenium inhibits tumor growth by inducing apoptosis of tumor cells and exhibits a dose-response relationship within a certain concentration range.

Keywords: Selenium, sarcoma, apoptosis

Introduction

Selenium is a trace element that is ubiquitous in the environment. Selenium is located in the fourth cycle of the chemical periodic table and the 34th position of the sixth family. It is one of the essential trace elements required for human life and has important biological effects on the body [1], such as participation in the synthesis of GSH-Px, protection of the structure and function of cell membranes, anti-oxidation, stimulation of immunoglobulin and antibody production, and reduction of certain toxic elements and substances; thus participating in the regulation of the tricarboxylic acid cycle, vision transmission and fertility.

Like other essential elements, the biological effects of selenium are bidirectional [2]. An excessive or insufficient intake of selenium in the human body can cause damage to human body [3, 4]. Lack of selenium can lead to increased incidence of hypertension [5], coronary heart disease [6, 7], diabetes [8], etc., and cause dysfunction of the thyroid [9] and immune system [10] as well as being related to the incidence of Keshan disease [11]. Conversely, excessive selenium intake can cause selenium poisoning [12].

In recent years, selenium has been reported to be involved in several malignant tumors [13, 14]. Related studies have shown that selenium can promote tumor cell apoptosis via regulating expression of oncogenes [15, 16] and exert anti-tumor effects by regulating the transcription pathway of tumor cell signaling [17], so selenium might have certain preventive and therapeutic effects on malignant tumors and can play a certain diagnostic role in malignant tumors. Therefore, the anti-tumor effect of selenium has received more and more attention and "selenium supplementation" has become a hot spot of current research.

About 51% of the soil in China is low in selenium content and the concentration of selenium in water, plants, soil and human diet is also low in most areas [18]. Therefore, with the current situation of insufficient intake of selenium, a reasonable and safe "selenium supplementation" has become an important problem to be solved. At present, nano-selenium, sodium selenite, and selenomethionine can be used as a source of selenium and the advantages of sodium selenite are cheap and convenient [19]. Therefore, this experiment intends to treat the S180 sarcoma mouse model with sodium sulphate and assesses selenium's effect on sarcoma, as well as the apoptosis rate of tumor cells to provide relevant theoretical basis for "selenium supplementation" to be used as anti-tumor therapy.

Materials and methods

Main reagents and instruments

Mouse sarcoma cell line S180 (Shanghai Institute of Cell Biology, Chinese Academy of Sciences); Kunming mice, 6 weeks old, weighing 18~22 g (Guangdong Animal Experimental Center); Clean Workbench (Suzhou Antai Air) Technology Co., Ltd., SW-CJ-IFD, low-speed centrifuge (Zhongjia Company, SC3614), electronic weighing scale (Shanghai Huade Weighing Apparatus Co., Ltd., ACS-6), electronic analytical balance (Sedore, BS224S, Germany), Optical microscope (Olympus BX40, Japan), flow cytometry (BD, FACS Calibur); sodium selenite (Beijing Zhonglian Chemical Reagent Factory), cyclophosphamide (Jiangsu Hengrui Pharmaceutical Co., Ltd., batch number 09-062521), The remaining reagents were all domestically analyzed.

Culture and inoculation of sarcoma S180 tumor strain

Nude mice were observed for 7 days to ensure their adaptation to the environment. Then S180 sarcoma cell line was inoculated into the peritoneal cavity of Kunming mice. On the 8th day after inoculation, the well-grown S180 sarcoma cells were injected. The ascites of mice was diluted 1:4 into sterile saline, and 0.2 ml was injected into the peritoneal cavity of Kunming mice to continue subculture. Passage was performed once for 2-3 d, and cells passaged for 7 days were used for inoculation on nude mice. A total of 112 healthy Kunming mice were used to establish a S180 sarcoma tumor-bearing mouse model. The ascites of S180 sarcoma tumor-bearing mice, which had been passaged for 7 days, were aseptically diluted in a clean bench and diluted with sterile saline at 1:4. 0.4% trypan blue staining, counting the number of viable cells under the microscope, and then adjusting the cell density to 1×10^7 cells/ml with sterile physiological saline, and inoculation of 0.2 ml/only in the right front of the shaved, conventional skin-sterilized mice. This study was approved by the animal ethics committee of our hospital.

Effect of sodium selenite on tumor growth

Twenty-four hours after inoculation of S180 sarcoma cells, mice were randomly separated into 8 groups (14/each group), blank control group (0.2 mL saline), positive control group (cyclophosphamide 20 mg/kg), and other 6 experimental groups treated with different concentrations of sodium selenite. Each group in the experimental group was injected with experimental drugs once per day according to the following requirements: group 1, sodium selenite 20 mg/kg; group 2, sodium selenite 40 mg/kg; group 3, sodium selenite 60 mg/kg Group 4, sodium selenite 80 mg/kg; Group 5, sodium selenite 100 mg/kg; Group 6, sodium selenite 120 mg/kg, continuous administration for 10 days. All mice were sacrificed on day 10 and 20, respectively, and the tumor was removed and weighed. An average tumor weight in the model group above 1 g indicates that the tumor grew well and can be evaluated for efficacy. Tumor inhibition rate (%) = (control group - experimental group average tumor weight)/control group average tumor weight × 100%. The tumor inhibition rate ≥30% is considered to have a certain anti-tumor effect.

Flow cytometry detection

Using mechanical separation plus enzyme digestion combined with gradient centrifugation, tumor tissue was made into a single cell suspension. Apoptosis rate was then measured by Annexin V/PI fluorescent staining by flow cytometry.

Table 1. Tumor weight of mice in different treatment groups

Group		Tumor weight (g)		t	P
		10th day	20th day		
Blank control		1.18±0.11	1.45±0.12	4.39	<0.01
Positive control		0.38±0.10	0.40±0.11	0.36	<0.36
Experimental	Group 1	0.97±0.13	1.13±0.12	2.39	0.02
	Group 2	0.76±0.09	0.90±0.11	2.61	0.01
	Group 3	0.63±0.08	0.72±0.09	1.98	0.04
	Group 4	0.57±0.07	0.63±0.06	1.72	0.06
	Group 5	0.49±0.12	0.52±0.10	0.51	0.31
	Group 6	0.41±0.11	0.44±0.13	0.47	0.32
	F	55.38	85.38		
	P	<0.01	<0.01		

Table 2. Tumor inhibition rate of mice in different treatment groups

Group		Tumor inhibition rate (g)		t	P
		10th day	20th day		
Blank control		-	-	-	-
Positive control		-	-	1.04	0.16
Experimental	Group 1	17.80±11.46	22.07±11.78	0.69	0.25
	Group 2	35.59±12.32	37.93±12.68	0.35	0.36
	Group 3	46.61±12.89	50.44±11.98	0.58	0.28
	Group 4	51.69±13.02	56.55±12.78	0.70	0.24
	Group 5	58.47±12.12	64.13±13.10	0.84	0.21
	Group 6	65.25±11.64	69.66±12.04	0.70	0.25
	F	17.10	17.37		
	P	<0.01	<0.01		

Table 3. Apoptosis of tumor cells in mice of different treatment groups

Group		Tumor cells apoptosis rate (g)		t	P
		10th day	20th day		
Blank control		8.72±1.78	8.90±1.81	0.19	0.42
Positive control		60.8±2.4	68.7±2.5	6.03	<0.01
Experimental	Group 1	19.8±2.3	20.4±2.6	0.46	0.33
	Group 2	21.7±2.7	23.5±3.1	1.15	0.13
	Group 3	28.6±2.9	33.9±2.7	3.54	<0.01
	Group 4	42.3±2.8	46.2±3.0	2.51	0.01
	Group 5	46.8±2.5	49.9±2.6	2.27	0.02
	Group 6	50.7±3.1	54.1±3.0	2.09	0.03

Statistical method

SPSS 19.0 software was adopted for analyzing data. The statistical distribution of normal distribution data was measured by mean ± standard deviation (SD) and assessed by t test or ANOVA. P<0.05 indicates a significant difference.

Results

Characteristics of experimental animals

There were no deaths in the two groups of mice. After inoculation with S180, mice in the blank control group ate less food, had less flexible movements, hair cyanosis, and poor mental state. The positive control group had better food intake, mental state and exercise status than the blank control group and the hair was smooth and shiny. The food intake and exercise status of the experimental groups were significantly improved, but slightly worse than the positive control group.

Effect of sodium selenite on tumor inhibition rate

The results of tumor weights were shown in **Table 1**. After injection of cyclophosphamide, the tumor weight was significantly reduced compared to control group, indicating that cyclophosphamide can significantly inhibit tumor growth. The weight of the tumors was decreased gradually with the increased concentration of sodium selenite in the experimental groups (P<0.05) and was lower than the blank control group (P<0.05). The tumor weight of group 1, 2 and 3 in the experimental group was higher than that in the positive control group (P<0.05). No statistical difference was found between average tumor weights of group 4, 5 and 6 in the experimental groups and the positive control group (P>0.05). Tumor weights on the 20th day in the control and experimental groups 1, 2, and 3 were significantly higher than that on the 10th day (P<0.05).

As seen in **Table 2**, the positive control group showed significantly inhibited tumor growth and the inhibition rate in the experimental group was increased with the increased concentration of sodium selenite (P<0.05). The

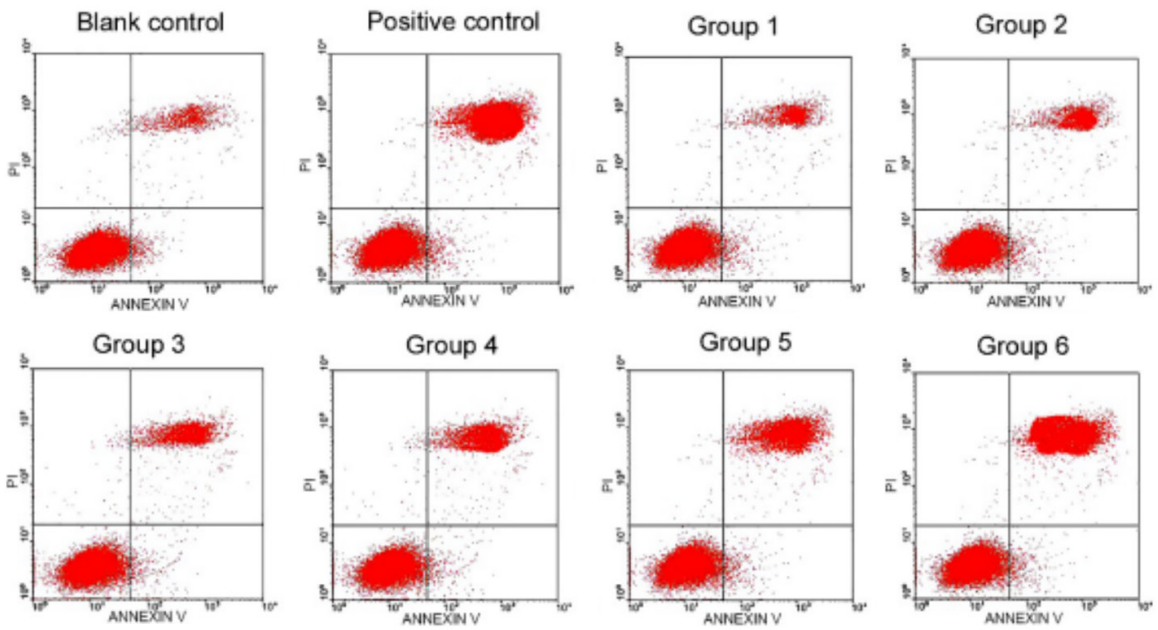

Figure 1. On the 10th day, the tumor cell apoptosis results of each group of mice.

inhibition rate on the 20th day was slightly higher than that on the 10th day with the prolongation of the administration time without significant differences (P>0.05).

Effect of sodium selenite on cell apoptosis

As shown in **Table 3** and **Figure 1**, the apoptotic rate of the positive control group and experimental group was significantly higher than control group (P<0.05), with lower rate in the experimental group than the positive control group (P<0.05). The apoptosis rate of the experimental group was gradually increased with the increased concentration of sodium selenite treatment. On the 20th day, the apoptotic rate of tumor cells in all groups was slightly higher than that on the 10th day. Apoptosis rate between positive control group and treatment group on the 10th and 20th day of the 3rd, 4th, 5th, and 6th groups showed significant differences (P<0.05).

Discussion

Selenium has a certain anti-tumor effect, and selenium supplementation can reduce the incidence of tumors [20]. Selenium can exert antitumor effects through cell cycle arrest and induce apoptosis [21, 22]. It is necessary to take a sufficient amount so that selenium can maintain a certain concentration in the blood to produce an anti-tumor effect. It can be applied to the treatment of clinical tumors; therefore reasonable, safe and effective "selenium supplementation" is an important issue that needs attention. At present, there are few studies on the standardization of "selenium supplementation". Therefore, different concentrations of selenium were used to treat the sarcoma mouse model in our study, followed by measuring tumor inhibition rate and apoptosis.

We found that with increased concentration of sodium selenite, tumor weight was gradually decreased and tumor inhibition rate was gradually increased. When the concentration of sodium selenite is 20 mg/kg, the inhibition rate of sodium selenite on mouse sarcoma is less than 30% which does not exert an anti-tumor effect. When the concentration of sodium selenite is ≥40 mg/kg, sodium selenite has a certain antitumor effect on mouse sarcoma, and this antitumor ability shows a dose-response relationship within a certain range. When the treatment concentration of sodium selenite was 120 mg/kg, its antitumor effect was close to that of cyclophosphamide, indicating that the high dose of sodium selenite was equivalent to the antitumor effects of cyclophosphamide. In the first phase clinical trial by Brodin O et al [23], the maximum tolerated dose of sodium selenite was found to be 10.2 mg/m^2, but only

the pharmacokinetics were explored, and the dose-response relationship was not reported. Sodium selenite can inhibit bladder cancer cell proliferation and enhance apoptosis in a dose-response relationship [24]. However, there is currently no report on the anti-tumor effects of sodium selenite in animal experiments and the dose-response relationship.

Induction of tumor cell apoptosis is an important mechanism of action of selenium against tumors. Therefore, this study further examined the apoptosis rate of tumor tissues. The results showed that the apoptosis rate in the experimental group was higher than in the control group. With elevated concentrations of sodium selenite, and the apoptosis rate increased gradually, showing a dose-response relationship, which further verified that selenium induces tumor cell apoptosis and exerts anti-tumor effects.

This study found that low concentrations of selenium have no significant anti-tumor effect. Higher selenium can inhibit tumor growth by inducing apoptosis of tumor cells, and exhibits a dose-response relationship within a certain concentration range. Treatment provides a theoretical basis. However, due to the insufficient concentration of selenium treatment in this study, if the concentration of sodium selenite is >120 mg/kg, whether the anti-tumor effect of selenium is more significant remains unclear and requires further research.

Conclusion

Selenium inhibits tumor growth by inducing apoptosis of tumor cells, and exhibits a dose-response relationship within a certain concentration range.

Disclosure of conflict of interest

None.

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