

INFLUENCE OF ZINC, MANGANESE AND SELENIUM ON SUPEROXIDE DISMUTASE ACTIVITY IN LUNG CANCER TISSUE AND CELL IN CULTURE

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In this experiment, the cancer tissues and cells, which were derived from Lewis lung cancer and A₅₄₉ lung cancer cell line, were respectively divided into four groups and zinc, manganese and selenium were respectively added to the medium for 24 hours. The superoxide dismutase activity in the tissues and the cells was estimated. It was found that the SOD activity was enhanced by zinc and manganese and the effect of zinc on SOD activity was superior to that of manganese. We supposed that the enhance of the SOD activity was relative to the activation of the SOD apoenzymes. This experimental result indicated that the inhibitory effect of zinc and manganese on carcinogenesis was achieved by SOD and the elements might be considered a SOD activator.

Key words: Superoxide dismutase, Zinc, Manganese, Selenium, Lung cancer, Tissue, Cell line, Culture.

As we know, zinc, manganese and copper are composition of superoxide dismutase (SOD). In many studies, it has been shown that in the patients with tumors the serum zinc and manganese level and the SOD content in erythrocytes were lower^{1,2} and at the same time the SOD activity was lower too in tumor tissues.³⁻⁶ Many investigations done by us and other researchers have been demonstrated that

zinc and manganese may inhibit the carcinogenesis induced by some chemical carcinogens in animals⁷⁻⁹ and reduce sister chromatid exchanges frequency in cancer cell.¹⁰ The effect of the two elements on tumors might be associated with SOD.⁸⁻¹⁰ In previous research, we found that zinc and manganese markedly increased the SOD in human renal cell cancer tissues in culture.¹¹ In this experiment, we investigated the influence of the additional zinc, manganese and selenium on SOD activity in lung cancer tissues and cells in culture in an attempt to further elucidate the relationship between these trace elements and SOD in the malignant process.

MATERIALS AND METHODS

Preparation of Cancer Tissues and Cells

The cancer tissues weighted about 30 grams was derived from Lewis lung cancer generated in mice and was cut into about 1 mm³ of tissue mass in size. The tissues were rinsed for three times in Hanks solution and divided into four groups at random. Then the tissues were cultured in flasks, which one flask included 5 masses of the tissues and every group consisted of 20 flasks, at 37 °C in humidified condition in 5% CO₂/95% air for 24 hours.

The cancer cells were A₅₄₉ lung cancer cell line, which was given as a gift by the Biology Department

of Wuhan University, and were cultured at the same condition.

Medium and Chemicals

Eagle MEM medium (Niesui, Japan) contained 15% foetal calf serum with penicillin G, streptomycin and amphotericin B. Chemicals were zinc sulfate, chloride manganese and selenium and stored in the concentrated solution of 10 mg/ml.

Experimental Groups

The cancer tissues and cells were respectively divided into four groups as follows:

Group control: the cultures were grown in the medium without zinc, manganese and selenium.

Group selenium: the cultures were grown in the medium with selenium which the final concentration was 1 µg/ml.

Group zinc: the cultures were grown in the medium with zinc which the final concentration was 1 µg/ml.

Group manganese: the cultures were grown in the medium with manganese which the final concentration was 0.1 µg/ml.

Harvest of the Cultures and Estimation of SOD Activity

After the cancer tissues were grown in the medium for 24 hours, 0.1 gram of the tissues were homogenized with 1 ml of 1.12% KCL. After homogenized, 0.25 ml homogenate was transferred to a test tube and 0.1 ml of 95% alcohol (4 °C) and 0.1 ml chloroform were added to the tube, and then was blended in mixer. After blended, the mixture was

centrifuged at 4,000 revolutions per minute for 5 min. The centrifugated fluid consisted of three strata. The upper stratum was supernatant fluid, which contained SOD, and was used for the assay of SOD activity. The fluid of the lower two strata was discarded.

The cancer cells grown for 24 hours were harvested by routine method and centrifuged. Then 0.1 gram of the cells were homogenized with 1 ml of 1.12% KCL too. The following extraction procedure was same as that of the tissues.

The SOD activity was determined according to the method of Ding Kexiang, et al.¹²

RESULTS

Zinc and manganese markedly enhanced the SOD activity in the lung cancer tissues and cells. The mean SOD activity in group zinc and group manganese was absolutely higher than that in group control and group selenium. The difference of the SOD activity was significant in statistics ($P < 0.01$).

The enhancing effect of zinc on the SOD activity in the tissues and the cells was superior to that of manganese. The SOD activity was 1638±61 U/g tissue wet and 343±29 U/g cell wet in group zinc, and was 1303±48 U/g tissue wet and 327±19 U/g cell wet in group manganese respectively. The SOD activity between the two groups was significantly different too ($P < 0.01$).

The SOD activity in the lung cancer tissues was much higher than that in the lung cancer cells in every groups.

The influence of selenium on the tissues and the cells was negative and the SOD activity in group selenium was similar to that in group control.

The detailed results were summarized in Table 1.

Table 1. SOD activity in lung cancer tissue and cell in four groups (ANOVA)

Group	SOD in cancer tissue			SOD in cancer cell			Comparison between two group	
	No. of sample	Range (U/g wet)	$\bar{x} \pm s$ (U/g wet)	No. of samples	Range (U/g wet)	$\bar{x} \pm s$ (U/g wet)	Groups	P
Control	29	550-1505	1132±50	20	370-631	505±38	Control and Se	>0.05
Se	30	690-1315	1104±45	18	381-529	499±31	Control and Zn	<0.01
Zn	30	993-1780	1638±61	23	502-712	677±40	Control and Mn	<0.01
Mn	30	851-1532	1303±48	20	510-647	581±37	Zn and Mz	<0.01

Se: selenium; Zn: zinc; Mn: manganese; SD: standard deviation;

DISCUSSION

It has been found that the SOD activity decreased in the cancer tissues and in the blood of patients with tumors. But how to increase SOD activity is still obscure. Because zinc and manganese are associated with many enzymes activity, therefore, it has been supposed that the inhibitory effect of zinc and manganese on the carcinogenesis might be realized by superoxide dismutase⁸⁻¹⁰ and the two elements might enhance the SOD activity. In this study, we found that zinc and manganese enhanced markedly the SOD activity in the cancer tissues and cancer cells. This result indicated that the effect of zinc and manganese on tumors was absolutely relative to SOD and demonstrated that the two trace elements might be considered a SOD activator. Lin Xinrong, et al. observed that manganese and selenium enhanced SOD activity of erythrocytes in mice *in vivo*.¹³

The lower SOD activity in patients with tumors may be associated with the lower zinc and manganese level in blood. However, zinc and manganese level in tumor tissues was higher than that in normal tissues, although serum zinc and manganese level was lower in patients with tumors.¹ Under these circumstances, why was the SOD activity in the tumor tissues lower than that in normal tissues? And why did the additional zinc and manganese enhance the SOD activity in the cancer tissues and cells? We supposed that lower SOD activity in tumor tissues was associated firstly with the reduction of mitochondria number in the cell¹⁴ and the existence of inactivated SOD apoenzymes and secondly with the presence of a combined zinc and manganese in tissues which might be utilized by SOD apoenzymes. Because of the two above reasons, the SOD activity in the tissue and the cells markedly enhanced after the additional zinc and manganese were added to the medium in this research. In our previously study, we observed that zinc and manganese enhanced SOD activity in human renal cell cancer tissue in culture.¹¹ Those investigative results showed that in cell there are some inactivated SOD apoenzymes, and under certain conditions those apoenzymes can be transformed into activated SOD.

The effect of zinc and manganese on enhancing SOD activity was superior to that of manganese, although the two elements all possessed the effect. The difference on SOD activity between the two elements might be relative to the content of Copper-

and zinc-containing SOD (Cu-Zn SOD) was higher than the content of manganese-containing SOD (Mn SOD) in tissues.¹⁴ Westman et al. reported that Cu-Zn SOD level was higher than that of Mn SOD in normal and tumor tissues.^{3-6,14} In addition, this experimental result showed that the SOD level in the tumor tissues was higher than that in the tumor cell line. We supposed that it was reason why the cancer tissues contained erythrocytes and interstitial tissues. Most reports demonstrated also that SOD activity in tumor tissues was higher than that in tumor cell line, although they possessed lower SOD activity.^{4,5}

Selenium, zinc and manganese all can inhibit the carcinogenesis in animals, but their mechanism is different. Selenium is composition of peroxidase and therefore it is not effective on SOD activity. Although a researcher observed that selenium enhanced SOD activity of erythrocytes in animal *in vivo*,¹³ we did not found the result at all.¹¹

Summarized the above results, in this experiment we observed that zinc and manganese markedly enhanced the SOD activity in lung cancer tissues and lung cancer cells and we supposed that the effect of zinc and manganese of SOD was achieved by increasing-concentration of the two elements in the cell and resulting in the activation of SOD apoenzymes. This meaningful result suggested zinc and manganese might be considered a SOD activator and supplement of appropriate zinc and manganese for patients with lower zinc and manganese level and lower SOD activity will be advantageous for the prevention and treatment of some tumors.

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