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Action of Selenium Supplemented to Drinking Water in the Prevention and Treatment of Oral Submucous Fibrosis in Mice

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ABSTRACT

The preventive and inhibitory effects of selenium (Se) in the drinking water on the submucous fibrosis in mice were studied in this experiment. Fibrosis was induced by injection of a 0.05 ml 4% phenol solution into the right oral submucosa. Forty-four ICR male mice were randomly divided into five groups. The drinking water of the control group contained no Se. The drinking water of groups 1 and 2 contained 2 and 4 ppm Se respectively after induction of fibrosis. The drinking water of groups 3 and 4 contained 2 and 4 ppm Se 48 days before fibrosis induction and throughout the experiment. The mice were sacrificed 14 or 28 days after induction of fibrosis. The specimens were taken and examined by autopsy and microscopy. The fibrotic thickness of the control group 14 days after fibrotic induction was significantly larger than that of the experimental groups. This demonstrated that addition of Se to the drinking water was effective in the prevention and treatment of submucous fibrosis. However, the fibrotic thickness of the control group 28 days after fibrotic induction was equal to or slightly larger than that of the experimental groups. And the difference was statistically insignificant. It might suggest that the longer period of oral exercise and tissue repair had contributed to the improvement of fibrosis in the control group.

Key words: Oral submucous fibrosis, selenium, drinking water, prevention, treatment.

Based upon previous research, a small amount of Se is essential to the human body. This is due to the multiple biological effectiveness of Se on the human body. it has been proven as an anti-carcinogen in an animal study[1], and could aid in the anti-carcinogenic therapy[2]. Se also acts as an antagonist of heavy metals poisoning such as As, Cd, Hg and Ag[3]. Se is considered essential for normal human growth and development[4]. It is an impor-

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tant component of glutathione peroxidase [5,6,7,8,9]. Se deficiency is closely related to development of coronary artery cirrhosis[10], liver cirrhosis[11], multiple cirrhosis[12] and arthritis[13,14]. It is also reported that Se deficiency was related to Keshen disease in the San-Si province of China and muscle pain syndrome in Finland and New Zealand[3,13,15].

A recent study reported that Se indirectly inhibits the formation of prostaglandin.Submucous fibrosis is the result of progressive inflammation of submucous scar tissue. The enhancement of Se may inhibit the inflammatory reaction caused by prostaglandin, and result in the prevention and inhibition of submucous fibrosis.The purpose of this experiment is to evaluate the effect of different Se concentrations on submucous fibrosis.

MATERIALS AND METHODS

1. Materials:

ICR male mice, 3 weeks after weaning and weighing 25 to 30 grams, were used in this experiment. The mice were placed in hardwood-chip lined plastic cages.

The laboratory was maintained at 21±1 degree C, 50% relative humidity, and the light toggled on/off for a 12-hour cycle to simulate normal conditions. Throughout the study, the animals were fed ad libitum with water and forage of Lab Chow 5001 (Charles River Co., Wilington, MA, USA). The drinking water was placed in a plastic bottle and changed twice a week. Na_2SeO_3 was bought from the Sigma Chemical Company, St. Louis, U.S.A.

2. Methods and Procedures:

The submucous fibrosis inducing method used in this experiment was adopted from Dr. Chen[18]. All mice in the study were anesthetized with a subcutaneous Ketamin injection into the abdominal area. 0.05 ml of 4% phenol solution was injected into the right oral submucosa to induce fibrosis.

Forty-four mice were randomly divided into five groups. The drinking water of the control group (consisted of nine mice) contained no Se throughout the experiment. The drinking water of group 1 (consisted of nine mice) contained 2 ppm Se after induction of fibrosis. The drinking water of group 2 (consisted of eight mice) contained 4 ppm Se after fibrosis induction. The drinking water of groups 3 and 4 (both consisted of nine mice) contained 2 and 4 ppm Se 48 days before the induction of fibrosis and throughout the experiment.

After the induction of fibrosis, the experiment was performed in two stages. In the first stage, 4 or 5 of the mice in each group were sacrificed by cervical vertebra luxation 14 days following the induction. In the second stage, the rest of the mice were sacrificed in the same way after an additional 14 days. The specimens were taken and examined by autopsy and microscopy after fixing with a 10% buffered formalin solution and staining with hematoxylin eosin and masson trichrome. The Student's t-test was used to compare the results of the control and experimental groups in this study.

RESULTS

This experiment has evaluated the preventive and inhibitory effects of Se on submucous fibrosis. The Studetn's t-test was used to examine the difference between control and experimental groups in each stage described previous-

ly.

In the first stage, the mean fibrotic thickness of the four experimental groups is significantly thinner ($p < 0.05$) than that of the control group (Fig. 1 and 2). Table 1 illustrates the experimental results for each group. This suggests that the addition of Se into drinking water was effective in preventing and inhibiting oral submucous fibrosis.

In the second stage, the experimental Group 1 has the same fibrotic thickness as the control group. The mean fibrotic thickness of the remaining experimental groups is thinner than that of the control group, but the difference is statistically insignificant ($p > 0.05$). Table 2 illustrates the experimental results for each group. Thus, the effectiveness of Se against submucous fibrosis is statistically insignificant after 28 days.

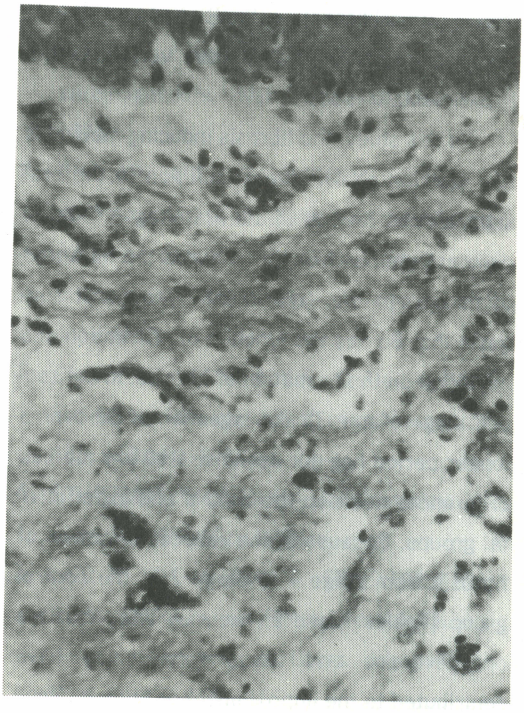

Fig. 1. (H.E. × 200) Group 1 showed marked fibrosis beneath epithelial layer which was intermingled with a few inflammatory cells (14 days after induction of fibrosis).

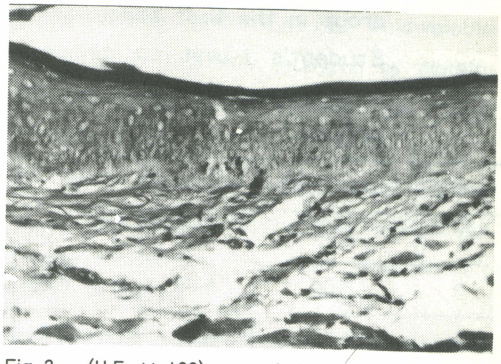

Fig. 2. (H.E. × 100) group 4 demonstrated narrower zone of fibrosis beneath epithelial layer (14 days after induction of fibrosis).

DISCUSSION

Oral submucous fibrosis is a progressive fibrotic disease in the oral cavity and oropharynx[16,17]. In the initial stage, the disease is characterized by an unusual sensation of the mouth and sensitivity to cold and heat. This is followed by difficulty with mouth opening. Epidermoid carcinoma is found in 5% of the advanced cases. The occurence of oral submucous fibrosis is related to environmental conditions, peculiar dietary components, and social customs.

Oral submucous fibrosis is not a common disease. Unfortunately, Tiawan is one of the countries with a high incidence of this disease. Betel-nut chewing, very popular in Taiwan, is closely related to oral submucous fibrosis. Almost all victims of the disease in Taiwan have the habit of chewing betel-nuts. However, the amount and frequency of betel-nut chewing are not proportional to the occurence of the disease. For instance, some victims of the disease have

Table 1. Mean fibrotic thickness of each group in the first stage, using Student's t-test to compare control and experimental groups.

group	experiment	control	p
1	4.75	6.60	$p < 0.05$
2	4.00	6.60	$p < 0.05$
3	4.50	6.60	$p < 0.05$
4	4.25	6.60	$p < 0.05$

Table 2. Mean fibrotic thickness of each group in the second stage, using Student's t-test to compare control and experimental groups.

group	experiment	control	p
1	4.75	4.75	$p > 0.05$
2	4.50	4.75	$p > 0.05$
3	3.75	4.75	$p > 0.05$
4	3.75	4.75	$p > 0.05$

only chewed a small amount of betel-nuts for a short time; while, some long time heavy chewers do not develop oral submucous fibrosis. Many scholars attribute this condition to the unique physique of each individual.

Fibrotic syndrome is usually associated with multiple oral epidermoid carcinomas. Se acts not only as an anti-inalammation but also as an anti-carcinogen[19,20,21,22,23]. The anti-inflammatory effect may be due to the indirect inhibition of prostaglandin formation by Se. Submucous fibrosis is the result of progressive inflammation of submucous scar tissue. The enhancement of Se may inhibit the inflammatory reaction caused by prostaglandin, and results in the prevention and inhibition of submucous fibrosis. If Se could be added to fruit and vegetable fertilizers, anti-inflammatory and anti-carcinogenic effects could be obtained after fortified vegetables and fruits have been ingested.

Lab Chow 5001 was utilized in this experiment. It contains protein, including methionine. It also contains 0.1 ppm Se, which corresponds to the needed Se concentration in food for a normal body. Therefore, the experimental result is not influenced by insufficient Se in the control group. The adequate amount of protein also increases Se absorption for the experimental groups. The estimated average volume of water drunk by each mouse is 4 ± 1 ml under constant temperature and humidity. Since the volume intake is nearly constant, the Se concentration is the priamry factor that causes variations the experimental results.

In this study, the fibrosis inducing method is the injection of 4% phenol solution to the oral submucousa. Results of the first stage (Table 1) demonstrated that Se has reduced the thickness of fibrosis in the experimental groups. It suggests that Se has the function of prevention and inhibition on the formation of fibrosis. In the second stage (Table 2), the ingestion of Se over a longer period has not demonstrated any marked differences between the control and experimental groups. Because of the reduced thickness of fibrosis from stage 1 to stage 2 in the control group (Tables 1 and 2), we may infer that the oral exercise and tissue repair may have contributed to the improvement of fibrosis. However, this condition does not occur in all experimental groups.

In this study, the increasing ingestion and

accumulation of Se in the body seem not to further improve the inhibitory effect on the formation of fibrosis. Likewise, oral exercise does not futher ameliorate fibrosis which has already been improved by previous ingestion of Se. Therefore, an in-depth investigation is recommended.

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飲水中添加硒對小白鼠口腔黏膜下纖維化的預防與治療作用

李勝揚　郭倍榮

硒是人體所必需的微量營養素，同時具有多重的生物效應。本實驗在飲水中添加亞硒酸鈉，依照不同的濃度和飲用時間，來觀察硒對小白鼠被誘發黏膜下纖維化的預防與抑制作用。

使用 ICR 小白鼠，將其隨意分成五組，對照組在飲水中不添加硒；第一組在黏膜纖維化誘發時，在飲水中添加 2ppm 硒；第二組在黏膜纖維化誘發時，在飲水中加 4ppm 硒；第三組在整個實驗過程中，添加 2ppm 硒，第四組在整個過程中，添加 4ppm 硒。

所有各組，皆在實驗開始後第 48 天，將小白鼠麻醉後，在右側口腔黏膜下，注射入 0.05ml 的 4% phenol，以誘導黏膜下纖維化形成，14 天後，所有各組的小白鼠均犧牲一半，經解剖及切片觀察，各組均有纖維化形成，而對照組較爲明顯；再 14 天後，犧牲其餘的小白鼠，亦經解剖及切片觀察結果實驗組與對照組無顯著差別。

本實驗顯示：飲水中添加硒劑對於預防及治療口腔黏膜下纖維化有減少之作用，而推測較長時間嘴巴運動有助於改善對照組的纖維化症，而使實驗組與對照組之結果無顯著差別。

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