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Effect of two selenium sources on hepatocarcinogenesis and several angiogenic cytokines in diethylnitrosamine-induced hepatocarcinoma rats☆

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ABSTRACT

This experiment was designed to compare the effect of two selenium sources at the dosage of therapeutic level on hepatocarcinogenesis and angiogenic cytokines in DEN-induced hepatocarcinoma rats to further approach their possible anticancer's mechanism. One hundred and seventy-eight Sprague-Dawley (SD) rats (average weight being 100–120 g) were randomly divided into 5 groups (I–V). Animals in group I, group II and group III served as the negative control, sodium selenite control (SS) and positive controls respectively, and received 0.1, 3.0, and 0.1 mg/kg selenium from sodium selenite supplemented diets during the whole experimental time. Rats in group IV and group V were fed with selenium from selenium-enriched malt (SEM) and sodium selenite (SS) supplemented diets (3 mg/kg respectively). To balance the nutritional content among each group, normal malt which was not treated with selenium was added into the diets of the challenge groups. The nutrition contents, except the selenium of the diet in each group, were similar and in accordance with NRC standards. Rats in groups III–V were treated by aqueous diethylnitrosamine solution (100 mg/L) at the dosage of 10 mg/kg body weight every day for 16 weeks to induce hepatocarcinoma, and drank sterilized water for an additional two weeks. Rats in group I and group II drank sterilized water throughout the experiment. At 4th, 8th, 12th, 16th week, five rats in each group were then sacrificed by cervical decapitation. At the termination of the study, at 18th week, the surplus rats were sacrificed by cervical decapitation. Feed was withheld from the rats for 12 h before sampling. The number of hepatoma nodules in liver and mortality of rats were calculated. The values of the following items, including α-fetoprotein (AFP), gamma-glutamyltranspeptidase (GGT), tumor necrosis factor-α (TNF-α), insulin-like growth factors-II (IGF-II), nitric oxide (NO) and total nitric oxide synthase (T-NOS) in plasma were determined. At the same time, the positive numbers of vascular endothelial growth factor (VEGF) and protein kinase C-α (PKCα) staining cells in tumor tissue were analyzed by immunohistochemistry using the Envision two step methods with a kit. The results indicated that SEM could significantly decrease the mortality of rats and the number of hepatoma nodules, values of GGT and AFP, and the levels of IGF-II, NO and NOS and lessen the positive numbers of VEGF and PKCα staining cells in tumor tissue. Moreover, SEM could increase the levels of TNF-α in the initiated time of hepatocarcinogenesis, whereas, decrease the levels of TNF-α in the progressive time of hepatocarcinogenesis. SS could only significantly inhibit the positive numbers of PKCα staining cells in tumor tissue, decrease the levels of GGT, AFP and TNF-α at minority sampling times, and increase the levels of NO. In conclusion, SEM could reduce the mortality. It might be related to deaden significantly the lesion of liver, delay the cause of hepatocarcinogenesis, and inhibit the progress of angiogenesis to increase the livability of DEN-induced hepatocarcinoma rats. SS at the same therapeutic dosage had less effect on the hepatocarcinogenesis by inhibiting angiogenesis and relative cytokines to some extent.

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Introduction

The trace mineral selenium is an essential nutrient of fundamental importance to human biology. Selenium is a component of selenoproteins, some of which have important enzymic functions [1]. It has now been recognized that all these enzymes are selenium-dependent, generally with selenocysteine at the active site. Selenium functions as a redox centre, for instance when the selenoenzyme, thioredoxin reductase, reduces nucleotides in DNA synthesis and helps control the intracellular redox state [2]. The best-known example of this redox function is the reduction of hydrogen peroxide and damaging lipid and phospholipid hydroperoxides to harmless products (water and alcohols) by the family of selenium-dependent glutathione peroxidases [3]. This function helps to maintain membrane integrity, to protect prostacyclin production and to reduce the likelihood of propagation of further oxidative damage to biomolecules such as lipids, lipoproteins and DNA with the associated increased risk of conditions such as atherosclerosis and cancer [1,4,5].

Large clinical trials have confirmed that an elevated selenium intake may notably be associated with reduced cancer risk or the development, dissemination and recidivation of cancer [6–8]. Moreover, the chemoprotective effect of selenium has been paid more attention due to the increase in cancer morbidity [6]. Different selenium compounds may have different efficacies in decreasing cancer morbidity and mortality. Due to the better preventive function than inorganic selenium, the chemoprotective effect of organic selenium has been paid more and more attention. Examining the effect of various level of dietary organic selenium has been the aim of few clinical trials in cancer prevention [9,10].

Tumor growth is angiogenesis-dependent and every increment of tumor growth requires an increment of vascular growth. Tumors lacking angiogenesis remain dormant indefinitely and rapid logarithmic growth follows the acquisition of a blood supply [11]. Angiogenesis is a strictly controlled process in the normal human body and is regulated by a variety of endogenous angiogenic and angiostatic factors [12]. Pathological angiogenesis often occurs in cancer, chronic inflammation, or atherosclerosis. Angiogenesis inhibitors can interfere with various steps in angiogenesis, while angiogenesis promoters can stimulate angiogenesis to occur basement destruction of blood vessels, proliferation and migration of endothelial cells. Tumor angiogenesis may highly correlate with tumor genesis, growth, metastasis and prognosis. Tumor cells induce formation of new blood vessels to supply the tumor mass with blood borne nutrients by a process termed “angiogenic switch” which is characterized by over expression of proangiogenic factors paralleled by decreased expression of antiangiogenic factors. Tumors lacking angiogenesis remain dormant indefinitely and rapid logarithmic growth follows the acquisition of a blood supply. The tumor angiogenic switch seems to be activated when the balance of angiogenic inhibitors to stimulators is shifted toward a proangiogenic milieu [11]. However a variety of factors, such as vascular endothelial growth factor (VEGF), tumor necrosis factor-α (TNF-α), insulin-like growth factors-II (IGF-II), PKCα (Protein kinase C-α), nitric oxide (NO) and total nitric oxide synthase (T-NOS) can interfere with angiogenesis [12,13].

High-dose selenium can inhibit angiogenesis and down-regulate the expression of VEGF in tumors [14] and down-regulate VEGF production by eliminating cancer cells both in vivo and in vitro [14]. In patients with cervix cancer, selenium level shows a negative correlation with TNF-α [15]. In mice, selenium also shows some functions with significantly decreasing the levels of TNF-α in a dose dependent manner [16]. Selenium polysaccharide krestin, as an organic selenium supplement, shows a significant function in promoting the release of NO in macrophages in rats [17]. All the above effects of selenium appear dose-dependent. Furthermore, cellular selenium can protect cells from NO-induced injury by inhibiting lipid peroxidation via changing membrane fluidity [18]. The results of our previous experiments had shown that selenium-enriched malt (SEM) had a better preventive function than sodium selenite on aflatoxin B_1-induced hepatocarcinogenesis in rats by enhancing antioxidants [19]. Moreover, SEM had more significant effects in reducing the number of hepatoma nodule in diethylnitrosamine (DEN)-induced hepatocarcinoma rats when compared with the same dosage of 3 mg/kg sodium selenite (SS) [20,21]. However, the two selenium sources with the same dosage of 3 mg/kg did not showed significant differences in delaying and reducing paraneoplastic hypoglycemia in DEN-induced hepatocarcinoma rats (except in the last week) [22]. It is necessary to compare further the difference of the two selenium sources in preventing cancer and their mechanism. Therefore, the present study was designed to observe the effect of the two selenium sources at the dosage of therapeutic level on hepatocarcinogenesis and angiogenic cytokines, including VEGF, PKCα, TNF-α, IGF-II, NO, NOS in DEN-induced hepatocarcinogenesis rats to further approach their possible anticancer’s mechanism.

Materials and methods

Selenium-malt

Selenium-enriched malt (SEM) was provided by The Institute of Nutritional and Metabolic Disorder in Domestic Animals and Fowls, Nanjing Agricultural University, China and manufactured according to the method reported by Li and Wang [23] and Zhu [24]. The selenium concentration of malt was 60.5 mg/kg dry matter. Ninety percent of Se existed in organic form and 60% Se existed within the protein [23,25].

Animals and treatments

This experiment was carried out on 178 Sprague-Dawley male rats (average weight being 100–120 g). The rats were supplied by the Shanghai Research Center of Experimental Animal of Academy in China and kept in conventional rodent room. Animals were caged in groups of five and offered food (Nanjing Jiangpu Feed Plant for Experimental Animal in China) and sterilized water. The temperature and relative humidity of the animal room were maintained at 21–24 °C and 40–60% respectively with 12 h light–dark cycles. The light cycle coincided with the day light hours. After 1 week of acclimation, the animals were assigned at random to the following groups:

Animals in groups I, II and III served as the negative control, SS control and positive control respectively, and received 0.1, 3.0, and 0.1 mg/kg selenium from sodium selenite (Sigma Chemical Co., St. Louis, Mo) supplemented diets respectively. Animals in groups IV and V were fed with selenium from SEM and SS supplemented diets respectively (3 mg/kg). To balance the nutritional content among each group, normal malt which was not treated with Se was added into the diets of the challenge groups. The nutrition contents, except the selenium of the diet in each group were similar and in accordance with NRC standards. Rats in groups III, IV and V were treated by aqueous diethylnitrosamine solution (100 mg/L) at the dosage of 10 mg/kg body weight every day for 16 weeks to induce hepatocarcinoma, and drank sterilized water for an additional two weeks. Rats in groups I and II drank sterilized water during the whole experimental time. The actual selenium level in the diet of each treatment was determined by AF-610A atomic fluorescence spectrometer (Ruili Analysis instrument Co., Beijing) with an electrically ignited concentric quartz tube atomizer. The actual selenium levels in the diets and treatments are reported in Table 1.