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Effect of selenium-enriched malt on hypoglycemia and regulatory hormones in diethylnitrosamine-induced hepatocarcinoma SD rats ☆

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ABSTRACT

One hundred and ninety-three Sprague-Dawley (SD) rats (average body weight being 100–120 g) were randomly divided into 5 groups (I–V). Animals in group I and group II served as the negative control and positive control, respectively, and both received 0.1 mg/kg selenium (Se) from sodium selenite. Animals in groups III–V were fed with Se from Se-enriched malt (SEM) supplemented diets (0.3, 1 and 3 mg/kg, respectively). Simultaneously, hepatocarcinoma were induced in groups II–V by diethylnitrosamine (DEN) solution (100 mg/L) at the dosage of 10 mg/kg body weight every day as drinking water for 16 weeks, then sterilized water for a further two weeks. Rats of group I drank sterilized water during the whole experimental time. 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 values of plasma glucose at different sampling times were measured. The values of the hormones in plasma related to plasma glucose metabolism, including insulin, glucagon, insulin-like growth factors-II (IGF-II), and the ratios of insulin/glucose (IGR_1), insulin/glucagon (IGR_2) and glucagon/glucose (GGR) were determined. At the same time, the correlation of plasma glucose concentrations related to hormones was statistically analyzed. The results indicated that the values of plasma glucose, insulin, glucagon and GGR in the groups treated with DEN were decreased significantly as compared with that of the negative control group, however, the values of IGF-II and IGR_2 were increased significantly. SEM showed a significant effect in suppressing the decreased of plasma glucose and glucagons, and delaying the increased of IGF-II and IGR_2 in the DEN-induced hepatocarcinoma rats. The plasma glucose concentrations revealed a significant relation to the hormones. In conclusion, SEM could reduce the development of hypoglycemia in the DEN-induced hepatocarcinoma rats by regulating the relative levels and balances or proportions of hormones.

1. Introduction

Paraneoplastic syndromes are disorders of organs or tissues that occur at a site distant from a primary cancer or its metastases (Yarbro et al., 1997). The first report of a paraneoplastic syndrome dates back to 18th century. The description of the relationship between neurological disorders and systemic tumors has been attributed to a French physician, M. Auchè, who described peripheral nervous system involvement in cancer patients in 1890. Paraneoplastic syndromes are rare disorders, even degenerative disorders, that are triggered by an altered immune system response to a neoplasm. They are defined as clinical syndromes involving non-metastatic systemic effects that accompany malignant disease. In a broad sense, these syndromes are collections of symptoms that result from substances produced by the tumor, and they occur remotely from the tumor itself. The symptoms may be endocrine, neuromuscular or musculoskeletal, cardiovascular, cutaneous, hematologic, gastrointestinal, renal, or miscellaneous in nature. It was well known that paraneoplastic syndrome, such as hypoglycemia and hypercalcemia were the obvious, primary and most important symptoms in human hepatocarcinoma with a morbidity of 30–100% and 4.26–10%, respectively (Liu and Wang, 2000). Although the tumor remained small, patients had probably suffered severe damage for a long time. Thus, these paraneoplastic syndromes had been operated as an important reference in the early diagnosis of tumor (Darnell and Posner, 2003; Albert and Darnell, 2004; Lalita et al., 2004; Jerome and Josep, 1997). It is important to recognize these paraneoplastic syndromes in the latter stages of hepatocellular carcinoma and treat them supportively to relieve pain and discomfort. In veterinary clinic, paraneoplastic

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hypoglycemia was also found in the dog and other animals (Eric et al., 2007).

Selenium (Se), an essential trace element and normal constituent of diets. Epidemiological, experimental, and clinical studies have shown chemopreventive potential for against cancer, diabetes mellitus, cardiovascular disease, white muscle disease, and so on (Combs et al., 2001; Raich et al., 2001; Lü and Cheng, 2001). Selenium appears to have a protective effect at various stages of carcinogenesis including both the early and later stages of cancer progression. Mechanisms for selenium-anticancer action are not fully understood; however, several have been proposed: antioxidant protection, enhanced carcinogen detoxification, enhanced immune surveillance, modulation of cell proliferation (cell cycle and apoptosis), inhibition of tumor cell invasion and inhibition of angiogenesis (Zeng and Combs, 2008). Many papers have demonstrated the protective effects of Se on cancer are strongly influenced by Se form (Ip et al., 2000; Davis et al., 1999). Some authors suggest that organic Se is an ideal additive for being absorbed and retained more than inorganic Se by animals and humans (Ortman and Pehrson, 1997). In contrast to selenite, the organoselenium compounds can be tailored to achieve greater chemopreventive efficacy with minimal toxic side effects by structural modifications (Howard, 1999). Any of these forms can support the nutritional requirements for the element; however, their bioefficacy depends on both dose and chemical form. At low doses, Se function is an essential component of SeCys in several specific selenoproteins and promote cell proliferation, a fact of particular importance to the immune response. At higher doses, but still nontoxic, Se can reduce cancer risk by stimulating tumor cell cycle arrest and apoptosis and inhibiting tumor cell migration and invasion (Zeng and Combs, 2008). Food is the major source of Se intake but limited efforts in common foods have been found (Bird et al., 1997a; Bird et al., 1997b). Supplementing dietary Se intake has been the aim of few clinical trials in cancer prevention (Lü and Cheng, 2001). With the rapid rise of the cancer morbidity, the protective of Se has been more clearly scrutinized (Popova, 2002).

The Se-enriched malt (SEM), as a organic Se supplement, showed greater bioactivity (such as increasing the activity of GSH-Px, and inducing metallothionein in heart, liver and kidney to show a cardioprotective effects) than sodium selenite in animals and humans (Xu et al., 2000; Li and Wang, 2004) and an ideal preventive function on the acute aflatoxin B_1-induced hepatocarcinogenesis (Liu et al., 2005) or the chronic diethylnitrosamine (DEN)-induced hepatocarcinogenesis (Liu et al., 2006) in rats. The effect of Se on the paraneoplastic hypoglycemia and the relative endocrine (including insulin, glucagon, insulin-like growth factor-II) change or modulation has not been investigated yet. As we known, insulin and glucagon are cooperated to regulate constant normal level of blood glucose. IGF-II also is an important hormone in the regulation of the blood glucose during the course of development of the hepatoma (Hunter et al., 1994). Therefore, the present experiment was designed to observe the possible effect of SEM in preventing paraneoplastic hypoglycemia and the main hormones regulating metabolism of blood glucose in DEN-induced hepatocarcinogenesis rats.

2. Materials and methods

2.1. Se-malt

SEM (selenium-enriched malt) was provided by 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 (2004) and Zhu (1999). The Se concentration of malt was 60.5 mg/kg dry matter. Ninety per cent of Se existed in organic form and 60% Se existed within the protein (Xu and Xiao, 1989; Li and Wang, 2004).

2.2. Animals and treatments

This experiment was carried out on 193 Sprague-Dawley male rats (average weight being 100–120 g). The rats were supplied by Shanghai Research Center of Experimental Animal of Academy in China and kept in conventional rodent room. Animals were caged in groups of five and given food (Nanjing Jiangsu Feed Plant for Experimental Animal in China) and sterilized water. The temperature and relative humidity of animal room were maintained at 21–24 °C and 40–60% respectively with 12 h light–dark cycles, the light cycle coinciding with the day light hours. After 1 week of acclimation, the animals were assigned at random to the following groups:

Animals in groups I and II served as the negative control and positive control, respectively, both received 0.1 mg/kg Se from sodium selenite (Sigma chemical Co., St. Louis, Mo) supplemented diets. Animals in groups III–V were fed Se from SEM supplemented diets (0.3, 1 and 3 mg/kg, respectively). 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 Se of the diet in each group, were similar and in accordance with NCR standards. Rats in groups II–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 Ding (Ding and Cai, 2002). Rats in group I drank sterilized water during the whole experimental time. The actual Se levels in diet of each treatment were determined by AF-610A atomic fluorescence spectrometer (Ruili Analysis Instrument Co., Beijing) with an electrically ignited concentric quartz tube atomizer (Pan et al., 2007). The results are reported in Table 1.

2.3. Sampling and samples analysis

All the rats were withheld diet for 12 h before sampling and humanely sacrificed by cervical decapitation. Conjunctival blood samples were collected before sacrifice and divided into two parts, one of which was anticoagulated with sodium heparin. The anticoagulating blood was subsequently centrifuged at 1500×*g* for 15 min and the plasma was collected. The levels of plasma glucose were determined by using kits (Yang and Qin, 1998).

The other half of blood sample was centrifuged at 1500×*g* for 15 min and the serum was collected and removed into disposable

Table 1
Se source, levels supplemented in diets, animals numbers and DEN treatment.

Treatment	Animal numbers	Se source	Se level (mg/kg)		DEN treatment
			Supplemental	Actual	
Group I (negative control)	25	SS	0.1	0.134 ± 0.002	–
Group II (positive control)	48	SS	0.1	0.129 ± 0.001	+
Group III(0.3 SEM)	40	SEM	0.3	0.330 ± 0.005	+
Group IV(1.0 SEM)	40	SEM	1.0	1.027 ± 0.008	+
Group V(3.0 SEM)	40	SEM	3.0	3.031 ± 0.010	+

Note: ① **SS** means sodium selenite; SEM means Se-enriched malt. "+" means treated with DEN; "–" means treated without DEN. ② Values are mean ± SD.