Effects of selenium and vitamin E deficiencies on level of leukotriene C_4*

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Abstract A reduction in the glutathion peroxidase (GSH-Px) activity of the blood and myocardium accompanied with an increase in the leukotriene C_4(LTC_4), free radicals and lipid peroxides (LPO) concentrations of the plasma and myocardium are found in Wistar rats fed with the low selenium (Se) and vitamin E (VE) grains from a Keshan disease (KD) endemic area for 11 weeks. The concentrations of LTC_4, free radical and LPO were decreased in these rats and the GSH-Px activity is strengthened except the group supplemented with VE alone. These results suggest that the dietary deficiencies of Se and VE might take part in the occurrence and development process of myocardial damage in KD and other ischemic and anoxic cardiomyopathy by affecting the activity of lipoxygenase in arachidonic acid metabolism and by accelerating the synthesis of LTC_4 owing to excessive free radicals and LPO.
Keywords Selenium, Vitamin E, Leukotriene C_4, Ischemic and anoxic cardiomyopathy

1 Introduction

Leukotrienes (LTs) is another group derivative of non-prostaglandin category produced in the metabolic way of arachidonic acid (AA) through lipoxygenase (LOas). Researches showed that LTs (LTA_4, LTB_4, LTC_4, LTD_4) possess extensive physiological effects and are concerned in these pathophysiologic processes about coronarospasm as well as myocardial ischemic diseases[1]. Some scholars abroad have observed that dietary trace element selenium (Se) and vitamin E (VE) may adjust the metabolism of AA and LOas and affect the biosynthesis of LTC_4[2−7]. But, we have not found any report about the effects of Se and VE on LTs and about effects of LTs on ischemic and anoxic cardiomyopathy up to this day. In this paper, we investigated the effects of dietary Se and VE on LTC_4 content and possible roles of LTC_4 in the ischemic and anoxic myocardial damage by feeding rats with the low Se and VE grains from the endemic area of KD (Keshan).

2 Materials and methods

2.1 Animals, diets and groups

From the animal laboratory in our university, weighing 80∼120 g, 54 young Wistar rats were divided into 6 groups according to body weight, with each group composed of a half femals and a half males. These groups were as follows: endemic grains (EG), EG+Se, EG+VE, EG+Se+VE, Non-EG and stock diet. The endemic grains were from the KD endemic areas of Shangzhi county in Heilongjiang province. Basic diets are composed of 0.89 corn, 0.10 soybean and 0.01 salt in mass fraction, and are replenished with vitamin AD 0.0496 g/kg (a kind of medical oral liquor, containing vitamin A 50 000 IU/g, Vitamin D 50 000 IU/g). The supplement of Se was 0.1 mg/kg (0.22 mg/kg sodium selenite) and VE content was 0.1 g/kg. The nonendemic grains were from the nonendemic area of KD in Changchun suburbs and their compositions were the same as those of EG. Composition of stock diet from the animal laboratory was: 0.48 corn, 0.20 soybean, 0.005 salt, 0.07 sorghun, 0.15 bran, 0.05 fish meal, 0.025 bone meal and 0.02 yeast in mass fraction.

2.2 Experimental methods

The quantities of diet and water were not limited during feeding the rats. At the end of an 11 week feeding period, the blood was collected through orbital vascular plexus to separate serum or plasma and the myocardium was obtained from the rats following their

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decapitation to prepare the homogenates according to different requirements for respective detection indices. Samples for detection indices were selected from the same anatomic site of myocardium in each rat. Se contents of endemic, nonendemic and stock diets were assayed with the aid of 2,3-diaminonaphthyl-fluorospectrophotometry and VE (α-tocopherol) content was determined by reserve HPLC. Glutathione peroxidase (GSH-Px) activities in the blood and the myocardium were determined by DTNB direct method. We determined free radical signal in myocardium tissue by using JESFE3AX electron spin resonance frequency spectrum instrument made by JEOL company, Japan, and calculated free radical concentration. Lipid peroxides (LPO) contents in serum were determined by the method of fluorescence spectrum and the LPO concentration in the myocardium was determined by TBA method. LTC_4 levels in the plasma and the myocardium were determined by using ^3H labelled RIA offered by Institute of Preclinical Medicine, the Chinese Academy of Medical Sciences. The single factor analysis of variance was used for the statistical analysis of the research results.

3 Results and discussion

Endemic grains contain Se 0.006 μg/g and VE 2.81 μg/g. Nonendemic grains contain Se 0.043 μg/g and VE 4.402 μg/g. Stock diets contain Se 0.160 μg/g and VE 8.98 μg/g.

Table 1 GSH-Px activity, free radical and LPO contents ($\bar{x} \pm s$)

Group	GSH-Px activity		Total free-radical contents	LPO contents	
	in blood/ $U \cdot L^{-1}$	in myocardium/ $U \cdot (g\ wet\ wt)^{-1}$	in myocardium/ $10^{14}\ spin \cdot (g\ wet\ wt)^{-1}$	in serum/ $\mu mol \cdot L^{-1}$	in myocardium/ $nmol \cdot (mg\ protein)^{-1}$
EG	10840±2010(7)	5.29±0.78(8)	3.449±2.429(6)	16.18±1.70(5)	18.525±1.159(6)
EG+Se	32980±6080(7)**	14.19±0.89(6)**	1.483±0.111(6)**	11.37±2.03(7)**	13.158±2.68(6)**
EG+VE	11460±1800(7)	5.34±0.93(8)	1.334±0.335(6)**	10.46±1.85(7)**	13.050±2.69(6)**
EG+Se+VE	33090±6670(8)**	14.60±2.82(9)**	1.291±0.465(6)**	11.06±2.23(7)**	12.197±2.18(6)**
Non-EG	13310±1490(7)*	7.04±0.90(7)**	1.734±0.595(6)*	11.81±2.47(7)**	12.485±1.09(6)**
Stock diet	33590±3360(7)**	10.83±2.08(7)**	1.067±0.149(6)**	9.56±2.57(7)**	10.084±3.34(7)**

*: $P < 0.05$, **: $P < 0.01$ compared with EG; (n): Number of cases

Table 2 LTC_4 concentration in plasma and myocardium of rats ($\bar{x} \pm s$)

Group	in plasma $/\mu g \cdot L^{-1}$	in myocardium $/ng \cdot (mg\ protein)^{-1}$
EG	1.573±0.840(6)	0.561±0.183(5)
EG+Se	0.889±0.419(6)**	0.370±0.048(5)*
EG+VE	0.715±0.169(6)**	0.408±0.028(5)**
EG+Se+VE	0.658±0.331(5)**	0.203±0.106(5)**
Non-EG	0.659±0.369(5)**	0.395±0.162(5)*
Stock diet	0.337±0.168(6)**	0.127±0.015(5)**

*: $P < 0.05$, **: $P < 0.01$ compared with EG; (n): Number of cases

From Tables 1,2, it can be seen that in the rats eating grains containing low Se and VE from KD endemic area, GSH-Px activities in blood and myocardium both decreased significantly; the myocardial free radical net contents and LPO concentrations in the serum and the myocardium as well as plasmic and myocardial LTC_4 contents all increased remarkably. It was observed when we supplied the endemic grains with Se or Se and VE that GSH-Px activities in the blood and myocardium both were raised; myocardial free radical net content and LPO concentration in the serum and the myocardium both lowered, especially plasmic and myocardial LTC_4 contents which were near to or even lower than those of Non-EG and stock diet groups. We also observed that in the group of adding sole VE to the endemic grains, all results except unchanged GSH-Px activity were consistent with those supplying Se or Se and VE. Our research results suggested that dietary deficiency of Se or/and VE could affect the metabolic way of AA and LDas, bring about some changes of the metabolic product LTC_4 content, might play a role in the occurrence and development of the myocardial damage in KD and other ischemic and anoxic cardiomyopathy. By adding Se or VE or Se and VE to the endemic grains, the free radical metabolic disorderly conditions in the rats could be improved and the production of LTC_4 in AA and LOas metabolism decreased; thus it might have a protective effect on myocardium.

In the past researches, author put forward that KD myocardial necrotic focuses surrounding small and fine arteries illustrate that some factors causing myocardium ischemia and anoxia might play a role in the process of KD developing. Some later studies demonstrated that the deficiencies of Se and VE might be basic cause of KD. Afterwards, author found that it is the peroxidation injury brought about by free radical metabolic disorder caused by the deficiencies of Se and VE being one of mechanisms of myocardial damage. But it is not very clear to the mechanism that how free radicals selectively affect myocardium; especially, KD is caused to have the myocardial necrosis of the ischemic and anoxic characters besides free radicals attacking directly on cytomembrane to cause membrane injury. It is known that LTC_4 has systolic function; can reduce myocardial contractility and accelerate platelet aggregation[8], inhibit the synthesis of prostacyclin (PGI_2), stimulate leukoagglutination and lysosome release[9]; possesses free radicals with cytotoxicity action. In this study we found that the LTC_4 content in the rats eating grains (low Se and VE) from KD endemic area increased, from which we can deduce reasonably that the rise in LTC_4 content might be involved in the development process of KD myocardial damage.

The researches began in recent years that how the changes of trace elements Se and VE in diet affect the metabolic products of AA and LOas. Dietary Se and VE can inhibit LOas activity[2~4]. The deficiency of Se can decrease GSH-Px activity, increase free radicals and LPO contents produced in metabolic processes of AA and LOas themself and other ones. If the intake of VE is insufficient, these functions of eliminating free radicals and stopping the chain reaction of lipid peroxidation could be weakened and also excessive free radicals and LPO produced, and the latter both have the effect to promote LTC_4 synthesis[5,6]. In addition, reduction in GSH-Px activity and obstacle of changing course 12-HPETE into 12-HETE in the process of the LOas metabolism caused the accumulation of 12-HPETE which promoted the production of LTC_4 and inhibited the synthesis of PGI_2 and PGI_2 can inhibit cells from secreting leukotrienes. Therefore, the decrease in GSH-Px activity accompanied with the increases in free radicals and LPO contents caused by dietary deficiencies of Se and VE taked part in the metabolism of AA and LOas and brought about excessive LTC_4 products by means of mechanisms and links above.

To sum up, ensuring adequate dietary intake of Se and VE is advantageous to diminish free radicals and LPO and especially the producing of LTC_4, which is one of the important measures in preventing and curing KD and other ischemic and anoxic cardiomyopathy.

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