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VITAMIN E DEFICIENCY HAS A PATHOLOGICAL ROLE IN MYOCYTOLYSIS IN CARDIOMYOPATHIC SYRIAN HAMSTER (BIO14.6)

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Summary: This study revealed the occurrence of vitamin E deficiency in the myocardium of 60-day-old Syrian cardiomyopathic hamsters (BIO14.6), and that this deficiency might be related to the increase in lipid peroxide. Vitamin E administration for ten days effectively restored creatininekinase activity and decreased the lipid peroxide content in the myocardium, returning these to normal control levels (F1b). These results indicate that vitamin E deficiency, possibly combined with oxidative stress in the early cardiomyopathic stage plays an important role in initiating the pathogenesis of myocardial lesions.

The cardiomyopathic Syrian hamster (BIO14.6) is an inbred strain with a hereditary abnormality in cardiac muscle and skeletal muscle, and constitutes a valuable model to study the pathological course and development of cardiac muscle diseases involving cytolysis, healing and compensating ventricular hypertrophy leading to heart failure. The earliest biological and morphological evidence of cardiomyopathy: cytolysis, usually appears by 30-40 days of age, and continues to maximal evidence by 60 days (1). It is believed that excess intracellular calcium may play an important role in the pathogenesis of the disease process, because the myocardium in this BIO14.6 model shows that calcium uptake into the myocardium is increased (2), cardiac action potentials demonstrate a prolongation compatible with an augmented voltage-dependent calcium channel (3), the number of calcium antagonist receptors is increased (4), and calcium antagonist drugs such as verapamil are effective in preventing the development of the disease (5-8). However, the underlying pathology through which the heart cells

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of the cardiomyopathic hamsters develop the calcium overload remains unknown. Recently it has been reported that exposure of the cardiac membranes to free radicals resulted in the alterations in the Ca^{2+}-transport system, involving increased permeability characteristics (9, 10), depressed Ca^{2+} pump activities (11, 12) and altered Na^{+}-Ca^{2+} exchange (13, 14). Similar alterations in the Ca^{2+}-transport system have also been reported in BIO14.6 cardiomyopathic hamsters (3, 4, 15, 16).

Vitamin E is known to be a antioxidant as well as a stabilizer of biological membrane (17), and experimental vitamin E deficiency in animals results in severe pathological conditions, including cardiomyopathy (18, 19). The purpose of the present study was to clarify the role of vitamin E and oxidative stress in initiating and developing cardiomyopathy in this model, and to demonstrate the protective effect of vitamin E on the myocytolysis.

MATERIALS AND METHODS

Experimental Animals

Male cardiomyopathic Syrian hamsters (BIO14.6 strain) at 50 days old, and age- and sex-matched male F1 hybrid healthy control Syrian hamsters (F1b) were obtained from Bio Research Consultants (Cambridge, U.S.A.). Both BIO14.6 and F1b hamsters were kept under identical conditions on a normal laboratory animal diet with tap water ad libitum.

To evaluate the effect of vitamin E on myocytolysis, the BIO14.6 hamsters were divided to two groups: The first group was administered 10mg/kg dl-α-tocopherol (Ea-0160-006, a gift from Eisai Co. Ltd., Tokyo: 100 mg dl-α-tocopherol, 200 mg polyoxyethylene hydrogenated castor oil derivatives 60 mole ether (Nikko Chemical Co. Ltd. Tokyo), 200 mg propylene glycol and 2 mg citrate in 2 ml distilled water) perorally daily for ten days, while the second group and the age-matched F1b were administered a vehicle (Ea-0160-106: Ea-0160-006 without dl-α-tocopherol) as controls.

Homogenate Preparation

After decapitation under diethyl ether anesthesia, the ventricles were quickly removed and immersed in ice-cold saline to avoid blood contamination. After removing connective tissue, vessels and fatty tissue, the ventricles were minced into small pieces, and after 10×dilution in ice-cold buffer (10mM sodium bicarbonate containing 5mM sodium azide (PH6.8)) were homogenized in a Brinkmann polytron homogenizer. The homogenate was then centrifuged at 1,000g for 10 min to remove debris. All procedures were carried out at 2-4 ℃.

Lipid peroxide assay of homogenate

The products of lipid peroxidation (thiobarbituric acid reactive substance: TBARS) in the organ homogenates were measured according to the procedure of

Ogura et al. (20) with slight modifications. TBARS were expressed as nanomoles of malondialdehyde (MDA) per mg protein.

α-Tocopherol assay of homogenate

The concentration of α-tocopherol was measured according to the method of Abe et al. (21) with slight modifications. Briefly the homogenate was saponified with KOH, and the mixture was extracted with n-hexane. Measurements of α-tocopherol were made using an Hitachi 655A-11 high-performance liquid chromatography (HPLC) equipped with an Hitachi F1100 fluorescence spectrophotometer (excitation wavelength, 290 nm; emission wavelength, 325 nm, and a YMC-PAC A-600(NH_2) column (50 mm by 4.6 mm; 3-mm particles; Yamamura Chemical Laboratories Co. Ltd., Tokyo)). The mobile phase, n-hexane/isopropanol (98/2, v/v), was run at a flow rate of 0.8 ml per minute. The content of α-tocopherol was calculated from the peak-height of fluorescence, using tocol as the internal standard and an authentic sample as the external standard (a gift from Eisai Co. Ltd., Tokyo).

Enzyme activities assay and protein assay of cardiac homogenate

Creatininekinase (EC 2.7.3.2, CK) was tested using an Iatrotech CK rate kit (Iatron Co. Ltd., Tokyo). The protein content was assayed with a Bio-Rad protein assay kit (Bio Rad Co. Ltd., U.S.A.), using bovine serum albumin as the standard. All assays were performed on fresh homogenates in an Hitachi 557 double beam spectrophotometer (Hitachi Co. Ltd., Tokyo) with adequate concentrations of organ homogenates within the region of linear dependence of the enzyme activity and the protein concentration.

Statistical Analysis

Values were expressed as mean$\pm$S.D., with any significant differences between groups, determined by Student's unpaired t-test. A value of $p<0.05$ was considered significant.

RESULTS

Lipid peroxide content and α-tocopherol content in the myocardium

Table 1(a) shows the lipid peroxide content by malondialdehyde formation (TBARS) in the ventricles. TBARS was significantly increased at 60 days of age.

Table 1(b) shows the α-tocopherol content in the ventricles. The BIO14.6 hamsters had a marked significantly smaller value than did the F1b, at 60 days of age.

Effect of the α-tocopherol administration on myocardium damage

As shown in Table 2, peroral vitamin E decreased the TBARS content and increased the CK activity in the ventricle homogenate, while inhibiting CK leakage into serum, and returned these values to the F1b control levels.

Table 1. α -tocopherol content and TBARS in ventricle homogenate from 60-day-old hamsters

BIO14.6	F1b	% control ratio	probability
a) TBARS in homogenate of ventricles (nmol MDA/mg protein)			
0.52 ± 0.07 (6)	0.37 ± 0.09 (6)	141	p<0.01
b) α -tocopherol content in homogenate of ventricles (ng/mg protein)			
10.4 ± 0.7 (6)	41.0 ± 8.6 (6)	25	p<0.01

Values are expressed as mean ± S.D., ( ): number of experiments.

DISCUSSION

The present study demonstrated the deficiency in α-tocopherol in the myocardium in the early cardiomyopathic stage associated with a concomitant increase in TBARS, and this TBARS level was sufficiently depressed by α-tocopherol administration over ten days, with restoration of the CK activity in the ventricles. It is known that an α-tocopherol deficient diet causes muscular dystrophy (22) and cardiomyopathy (18, 19) in animals. The present study is the first report of α-tocopherol deficiency in cardiomyopathy with a normal diet. α-Tocopherol is a lipid-soluble, chain-breaking antioxidant capable of scavenging oxygen-centered free radicals, as well as, being a stabilizer of biological membrane (17), and the endogenous α-tocopherol is depleted by oxidative stress (23, 24). The ability to take up α-tocopherol into myocardium was intact in BIO14.6, with no difference in α-tocopherol content between BIO14.6 and F1b before 30 days old (data not

Table 2. Effects of α -tocopherol administration on myocardial damage

	BIO14.6 with V.E [1]	BIO14.6 with vehicle [2]	F1b with vehicle [3]	probability	
a) TBARS and CK activitiy in ventricle homogenate				1)-2)	1)-3)
TBARS	0.33 ± 0.07 (6)	0.60 ± 0.05 (6)	0.40 ± 0.04 (6)	p<0.01	N.S
CK	10300 ± 650 (6)	9040 ± 650 (6)	11230 ± 620 (6)	p<0.01	p<0.05
b) CK activity in serum					
CK	4110 ± 800 (6)	7500 ± 1180 (6)	112 ± 18 (6)	p<0.01	p<0.01

Values are expressed as mean ± S.D., ( ): number of experiments, N.S: not significant
V.E: Ea-0160-006, vehicle: Ea-0160-106.

shown). The α-tocopherol deficiency in BIO14.6 might therefore caused by α-tocopherol consumption by free radicals production in the myocardium. The α-tocopherol deficiency and the preceding free radical production in the myocardium could synergically enhance a calcium overload through disturbing the Ca^{2+}-transport system in membrane with loss of integrity, resulting in the remarkable myocytolysis in the early cardiomyopathic stage. Recently, Jasmall et al. (25) reported that dietary selenium prevented oxidative injury in this animal and indicated the importance of oxidative stress, although they did not investigate either vitamin E or selenium deficiency. Vitamin E deficiency is known to be partially replaceable by selenium (26), and the effect of selenium can effectively be interpreted from our report of vitamin E deficiency in this model.

The underlying mechanism by which the heart cells of cardiomyopathic hamsters evoke free radicals remains unknown, although vitamin E administration effectively prevented myocytolysis and prevented an increase in lipid peroxides. Our results are evidence that vitamin E deficiency, possibly combined with oxidative stress, in the early cardiomyopathic stage plays an important role in initiating the pathogenesis of myocardial lesions.

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