Effect of α-Tocopherol on the Production of Malondialdehyde in Rat Tissue Homogenates after Hypobaric Exposure

Z. Z. VARŠKEVIČIENĖ, R. Č. ČERNIAUSKIENĖ and P. S. GRYBAUSKAS

Kaunas Medical Institute, Institute for Cardiovascular Research, 4 Eiveniu st., 233007 Kaunas, Lithuanian SSR, USSR

Abstract. Alpha-tocopherol content and production of malondialdehyde (MDA) was measured in liver, kidney, heart, lung, brain and skeletal muscle homogenates of control and hypoxic rats (following a 2-h-exposure to 200 mm Hg): the samples were incubated at 37 °C in air for 1 h. MDA production showed no relation with the content of α-tocopherol in control and hypoxic rats. In control animals, the lowest MDA level was found in lungs: it was several fold lower than in other tissues. After hypobaric exposure, a marked increase in MDA level could be observed in lungs only. No marked changes in α-tocopherol concentration could be observed in any of the tissues tested. A single i. p. injection of 25 and 50 mg-/α-tocopherol acetate/kg body mass, 2 hours prior to the exposure produced organ-specific accumulation of α-tocopherol. Both doses of α-tocopherol resulted in a reduced (by about 40 %) production of MDA in lung homogenates. The addition of α-tocopherol (750 nmol/g wet tissue mass) to homogenates from control and hypoxic rats prior to the incubation resulted in a marked inhibition of MDA production in all tissues (49—70 %).

Key words: α-tocopherol — Malondialdehyde — Hypobaric hypoxia

Introduction

Increased lipid peroxidation in membranes can be observed at both high or low tissue oxygen levels. Accumulation of toxic metabolites, such as lactic acid, denaturated proteins and products of lipid peroxidation, is assumed to be the cause of the ischemic cell damage. It has been suggested (Kogan et al. 1976; Chumakov et al. 1979; Demopoulos et al. 1980) that the main causes of intensification of the lipid peroxidation at low oxygen tensions are a decrease in the activity of antioxidants, and release of activators from stores.

α-tocopherol prevents lipid peroxidation-mediated damage to the cellular membranes. It was shown that the administration of α-tocopherol to experimental animals markedly increased the survival rate of animals subjected to local organ ischemia (Kogan et al. 1976; Takenaka et al. 1981; Bilenko 1982), and total acute

hypoxia (Aidarkhanov et al. 1978; Yakhnina 1980).

The effectiveness of α-tocopherol is usually related to its local concentration; however the great majority of studies have failed to assess the tissue tocopherol content. The objective of our study was to determine (1) the α-tocopherol content in rat tissues after a hypobaric exposure, and (2) the effectiveness of two parenteral single doses of α-tocopherolacetate upon lipid peroxide production on tissue homogenates. Since a periodic hypobaric exposure is known to be an adaptive measure which can decrease cell damage under stress (Meerson et al. 1982), examination of this kind of hypoxia was considered to be of obvious interest.

Materials and Methods

Fourty five mongrel white male rats weighing 150—250 g were randomly assigned to four groups: one control (C) and three experimental (H_0, H_1, H_2) ones. The experimental animals were exposed to hypobaric conditions (200 mmHg) for 2 hours. α-tocopherolacetate was administred to animals of both the H_1 and H_2 groups intraperitoneally by a single injection (H_1: 25 mg/kg body mass, H_2: 50 mg/kg) 2 hours prior to the hypobaric exposure. Both groups C and H_0 animals were injected with physiological saline.

The content of α-tocopherol and malondialdehyde, a secondary product of lipid peroxidation, was assessed in liver, kidney, heart, lungs, brain and skeletal muscle homogenates. Following decapitation, the organs were rapidly removed and immersed in liquid nitrogen. Frozen organ samples (300 mg) were homogenized in 4.6 ml of 0.025 $mol.l^{-1}$ tris-buffer (pH 7.5) supplemented with 0.175 $mol.l^{-1}$ KCl.

α-tocopherol concentration was assayed by the *fluorometric method* (Taylor et al. 1976). MDA levels were determined following incubation of homogenates in air for 1 hour at 37 °C (control samples). The reaction was stopped by additing 1 ml of 17 % trichloracetic acid. Experimental samples were prepared in parallel to the control ones, the only difference being the addition of α-tocopherol (750 nmol/g wet tissue mass) to the homogenates prior the incubation. MDA concentration was estimated by the thiobarbituric acid test, using fluorometric measurements (Tanizawa et al. 1981). Fluorescence intensities of both α-tocopherol and the reaction product of MDA with the thiobarbituric acid were measured using a Hitachi MPF 2A spectrofluorometer.

Results

Fig. 1 summarizes results of MDA levels obtained in tissue homogenates of control and hypoxic rats without and after α-tocopherolacetate administration. In control animals, the lowest MDA production was found in lung homogenate, while the highest levels were observed in liver and brain samples, the latter two exceeding the lung level more than fourfold. After hypobaric exposure, MDA levels in the lungs were approximatly threefold increased as compared with the control levels. In other tissues studied a nonsignificant decrease was noted.

Table 1 summarizes the results of α-tocopherol measurements. Higher α-tocopherol contents were found in lung, heart and brain samples as compared with liver, kidney and skeletal muscle homogenates from control animals. Hypobaric exposure did not result in marked alterations of the content of

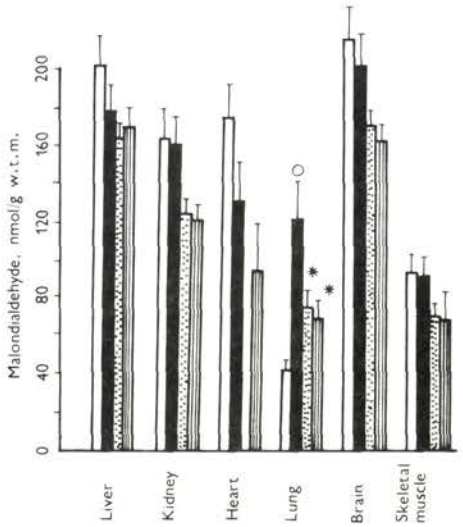

Fig. 1. Intensity of lipid peroxidation in tissue homogenates from control and hypoxic rats without and after α-tocopherolacetate administration. Mean ± S. E. of MDA formed over 1 hour at 37 °C in air are given: (empty column = C, full column = H_0, dotted column = H_1, dashed column = H_2 groups). Empty circle indicates the significance of the difference versus control value, the significance versus hypoxic (H_0) value ($P < 0.05$) is indicated by asterisk.

Table 1. α-tocopherol concentration in tissues of control and hypoxic rats without and after α-tocopherolacetate administration.

Tissue	α-tocopherol, μmol/kg w. t. m. (mean ± S.E.)			
	Groups			
	C	H_0	H_1	H_2
	n = 16	n = 12	n = 8	n = 9
Liver	20.2 ± 4.4	23.5 ± 3.9	154.2 ± 21.3***	320.0 ± 37.4***
Kidney	27.7 ± 2.1	24.6 ± 3.7	152.3 ± 19.1***	190.2 ± 18.1***
Heart	36.3 ± 2.6	42.8 ± 3.7	—	131.6 ± 18.6***
Lungs	39.1 ± 3.0	38.4 ± 4.2	59.1 ± 5.8**	76.3 ± 10.0**
Brain	34.4 ± 3.2	34.6 ± 2.6	32.3 ± 3.5	37.0 ± 4.4
Skeletal muscle	27.2 ± 2.1	27.7 ± 3.7	69.5 ± 11.4***	133.9 ± 13.2***

Asterisks indicate the significance of differences versus control value (**$P < 0.01$, ***$P < 0.001$) α-tocopherol was not assayed in hearts from rats of the H_1 group.

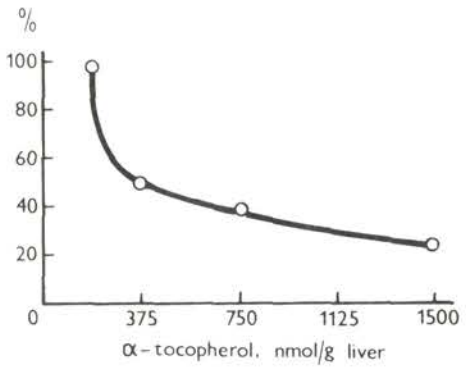

Fig. 2. Effect of the addition of α-tocopherol to liver homogenates of hypoxic rats on MDA production. Each point represents the average of 4 tissue samples expressed in per cent of the average of 4 control samples.

α-tocopherol. No oxidation of α-tocopherol occurred during the incubation of tissue homogenates. As pointed out by the authors of the α-tocopherol assay this method allows monitoring of tocopherol oxidation. In our experiments, α-tocopherol concentration in incubated homogenates was 98.7 ± 5.4 % of that before incubation (results from three control rats after the administration of 25 mg/kg α-tocopherolacetate). MDA production showed no correlation with α-tocopherol levels in control and hypoxic (H_0) groups.

The administration of α-tocopherolacetate resulted in a marked dose-dependent elevation of α-tocopherol concentrations in all the tissues examined but the brain (Table 1). Tocopherol accumulation was organ-specific: the liver showed the highest level (6.3 fold-group H_1, and 13 fold-group H_2, that in group H_0), with the level in lungs exceeding control levels (group H_0) 1.5-fold in group H_1 and 1.9-fold in group H_2. The administration of α-tocopherolacetate led to an inhibition of MDA production in lung homogenates of hypoxic animals by about 40 %: there was no detectable difference between the effect of the two doses used. Inhibition of MDA formation in other tissues was insignificant.

A relationship between the concentration of α-tocopherol and MDA levels could be shown in in vitro experiments. Fig. 2 illustrates data concerning lipid peroxidation in liver homogenates, preincubated with α-tocopherol. The inhibitory effect of α-tocopherol becomes evident at concentrations exceeding 370 nmol/g wet liver mass. The inhibitory effect of α-tocopherol was studied in vitro in tissue homogenates from control and hypoxic rats. The addition of 750 nmol/g of α-tocopherol wet tissue mass to the homogenates prior to their incubation in air

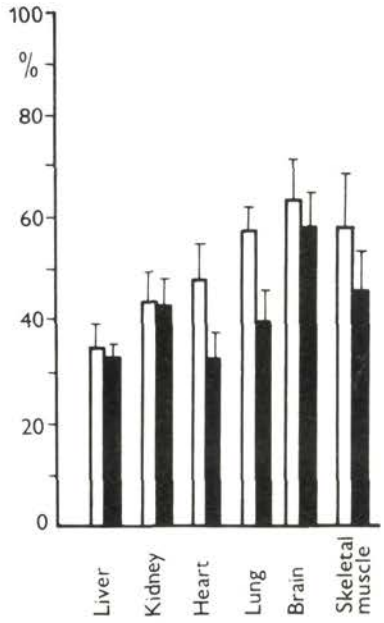

Fig. 3. MDA production in tissue homogenates preincubated with 750 nmol/g wet tissue mass α-tocopherol (experimental samples). Mean values ± S. E. of 16 control (□) and 12 hypoxic (■) rats are given.

resulted in an inhibition of MDA production in all the tissue homogenates (by 40—60 % as compared with controls). A significant difference in the extent of inhibition of MDA production between tissues from control and hypoxic animals was typical only of the lungs and heart: the inhibitory effect was more expressed following hypoxia. Brain homogenates were the most "resistant" tissue against the α-tocopherol effect in vitro.

Discussion

MDA production was estimated in hypoxic rat tissue homogenates in vitro. MDA determination in fresh homogenates is troublesome because of probable artefacts due to sample preparation. It is no clear, whether post incubation MDA concentration correlates with in vivo peroxidation or not; but it has however been suggested that in vitro lipid peroxidation data do also reflect lipoperoxidative capacity of tissues. Determination of MDA in incubated homogenates enables a comparison of MDA and α-tocopherol levels after α-tocopherolacetate administration irrespective of the time-dependent distribution of this antioxidant in various tissues.

The evidence obtained showed that a two-hour exposure of rats resulted in a significant increase in the in vitro formation of MDA in the lung only. MDA production in lungs of control animals was several fold less than that in homogenates of other tissues. It has been reported that tocopherol concentration in microsomal membranes from different rat tissues varies dramatically according to the apparent need of the particular tissue for antioxidant protection (Kornbrust and Mavis 1980). Thus the lungs, the most intensely oxygenated tissue, contained the highest level of microsomal tocopherol, approximately 6 times the tocopherol level in liver and brain, and about twice that of the heart microsomes. These data can explain the „resistance" of the lung homogenates to the production of MDA. Acute hypoxia however caused a damage to the systems protecting against lipid peroxidation, thereby increasing dramatically MDA production in the lung homogenate of hypoxic animal, leaving the level of lipid peroxidation in the liver, brain and heart tissue unaffected. Our results have showed that hypobaric exposure did not alter the α-tocopherol content in the tissue studied. Also studies in newborn rats did not reveal any significant changes in the lung tocopherol concentration resulting from an exposure to hyperoxia (Bucher and Robersts 1981). Animals can likely maintain this tissue store even in spite of some dynamic processes. Thus the primary cause of intensification of lipid peroxidation under acute hypoxia is a decrease in the activity of protective enzymes, such as superoxide dismutase, than a decrease in the content of the antioxidant α-tocopherol (Chumakov et al. 1979) rather.

The administration of α-tocopherolacetate prior to hypobaric exposure also abolished the intensification of lipid peroxidation in lung homogenates, leaving other tissues almost unaffected, although the accumulation of this antioxidant in the lungs was several fold less than that in liver, kidney or heart respectively. Thus α-tocopherol mainly inhibited lipid peroxidation induced by hypoxia. The fact that the in vitro inhibitory effect of α-tocopherol on MDA production is similar in all tissues can be explained by considerably higher doses used (about 30-fold) as compared to the levels of this antioxidant present under physiological conditions.

Our results suggest that the increase in the survival rate of experimental animals exposed to hypobaric conditions, due to pretreatment with α-tocopherol, should be brought in relation with a decreased lipoperoxidative potential in lungs rather than with that in other tissues.

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