Free Rad. Res., Vol. 26, pp. 169–174
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Increase of Lipid Hydroperoxides in Tissues of Vitamin E-Deficient Rats

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(Accepted by Prof JMC Gutteridge)

(Received 23 July 1996; In final form 28 August 1996)

The level of lipid hydroperoxides was determined by a newly developed method in rat tissues of vitamin E deficiency, which was a good *in vivo* model of enhanced radical reactions. In the heart, lung and kidney, the level of lipid hydroperoxides increased significantly as early as 4 weeks after feeding on a tocopherol-deficient diet compared with that of the control group. After 8 weeks of the deficiency, similar results were obtained. These results indicate that the lipid hydroperoxide is available as an extremely sensitive indicator of lipid peroxidation in these organs, because it takes several months to detect manifestations of the vitamin deficiency based on conventional indices.

Keywords: Vitamin E deficiency; tocopherol; hydroperoxide; vitamin E; peroxide; lipid hydroperoxide

Abbreviations: GSH, glutathione; MDA, malondialdehyde; SOD, superoxide dismutase, TBARS, thiobarbituric acid-reactive substances

INTRODUCTION

Although radical reactions receive much attention in relation to pathogenic mechanisms such as atherosclerosis,[1] cancer,[1] ageing[2] and so on,[1] the search for a satisfactory indicator of lipid peroxidation is still the central subject of the field. Conventional indicators are classified in three main categories which are; *products of lipid peroxidation* such as malondialdehyde (MDA),[3] thiobarbituric acid-reactive substances (TBARS),[4] modified proteins[5] and DNA,[6] *decreased antioxidants* such as vitamin E,[7] GSH (glutathione)[8] and ubiquinol[9] and *activity change of antioxidant enzymes* including SOD (superoxide dismutase)[10] and glutathione peroxidase.[11] As another kind of index, the oxidative mediator, rather than the products of peroxidation, may be postulated. Lipid hydroperoxide is a probable candidate for such an oxidative mediator, because it is formed by radical reactions, has sufficient lifetime to migrate and generate finally reactive radicals to peroxidate protein and DNA.

The determination of lipid hydroperoxides has been hampered by their instability and extremely low level. The chemiluminescent detection[12] of hydroperoxides combined with the separation of the lipid components with HPLC is very sensi-

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tive, but cannot be applied to the most fundamental determination, i.e., to the quantitation of total hydroperoxides in biological samples, because endogenous compounds such as ubiquinol and tocopherols interfere seriously with the chemiluminescence reaction.[13]

Recently we developed a specific and sensitive method[14] to determine the level of lipid hydroperoxides in animal tissues involving chemical convertion of 1-naphthyldiphenylphosphine into its oxide with lipid hydroperoxides followed by the measurement of the oxide with HPLC. In this paper, we report the increase of lipid hydroperoxides in the tissue of vitamin E-deficient rats. Since α-tocopherol is the most important lipid-soluble antioxidant, its deficiency may be a good model to examine the efficiency of indices of lipid peroxidation *in vivo*.

MATERIALS AND METHODS

Materials

1-Naphthyldiphenylphosphine and its oxide were prepared according to the literature.[14] Authentic tocopherols were purchased from Eisai Co. Ltd. (Tokyo). All other reagents were of analytical grade and were purchased from Wako Pure Chem. Co. Ltd. (Osaka).

Animals and diets

A notice of the Prime Minister's Office of Japan (No. 6 of 27 March 1980) for the care and use of laboratory animals was followed. Three week-old rats (strain, Slc:Wistar) were obtained from Japan SLC (Hamamatsu, Shizuoka). The animals were housed in a room with a temperature 24 ± 2°C, and a 12-h light dark cycle. Animals were permitted free access to food and water. For the first week, all rats were supplied with a synthetic basal diet (AIN 76;[15] recommended by the American Institute of Nutrition and provided by Oriental Yeast Co. Ltd., Tokyo). Proximate composition of the diet was as follows: vitamin-free casein (20.0%). DL-methionine (0.3%), cornstarch (15.0%), sucrose (50.0%), fiber (5.0%), corn oil (5.0%), AIN mineral mix[15] (3.5%), AIN vitamin mix[15] (1.0%) and choline bitartrate (0.2%). The diet contained vitamin E (50 mg/kg diet) and did not contain vitamin C.[15]

After the week of acclimation, rats were divided into 2 groups. The control group received the same diet and the experimental group was fed with vitamin E-deficient diet (as a fat, vitamin E-free lard was used instead of corn oil) which was purchased from Oriental Yeast Co. Ltd., Tokyo.

Diets were divided into small portions and stored at −85°C until use.

Analytical methods

The level of hydroperoxides in rat organs was determined according to the method.[14, 16] The level of α-tocopherol was determined on the literature[17] using HPLC (Shimadzu, LC-10A) equipped with a fluorescence detecter (Shimadzu, RF-535, Kyoto). The conditions of HPLC and detection were reported previously.[7] TBARS were measured as described[4] and expressed as nmol equivalent of MDA/g tissue.

For the determination of GSH, the tissue was treated according to the literature[18] and glutathione was determined by the enzymatic method.[19] The activity of glutathione peroxidase (EC 1.11.1.9) was measured based on the literature[20] using cumene hydroperoxide, which was dissolved in 25% (v/v) methanol at 30 mM (i.e., the final concentration in the assay tube was 3 mM), as a substrate. The activity was expressed as the decrease of NADPH/mg protein/min. The activity of superoxide dismutase (SOD: EC 1.15.1.1) was determined and expressed as nitrite units (NU)/mg protein according to the literature.[21] Protein concentrations were determined according to the method[22] using bovine serum albumin as the standard.

Data were expressed as mean ± SD and analyzed statistically by Welch's *t*-test.

RESULTS AND DISCUSSION

Vitamin E deficiency for 4 weeks

The content of α-tocopherol in the brain, heart, lung, liver, kidney and muscle of the deficient animals which were fed with vitamin E-deficient diet for 4 weeks, were all significantly lower than the control as shown in Table I. Other tocopherols (β, γ and δ) were not detected in the present experiment. The decrease of tissue vitamin E in the present experiment was much more rapid compared with the literature,[23] where the deficient experiment was performed using 60 day-old rats. The difference may be explained on the different storage of vitamin E on maturation. On the other hand, the decrease of vitamin E in the liver of vitamin E deficient rat using weanling rat[24] was similar to the present study.

The hydroperoxide levels in the heart, lung and kidney of the deficient animals were significantly higher than those in the corresponding organ of the control rats as shown in Table I. This result demonstrates that lipid peroxidation is enhanced and that the oxidative mediator represented by hydroperoxide increases in these organs after as short as 4 weeks of the vitamin deficiency. These observations indicate that the level of hydroperoxides is available as a sensitive indicator of oxidative stress in these tissues, since it is well known that it takes several months to detect manifestations of vitamin E-deficiency.[26] TBARS which are used conventionally as an index of lipid peroxidation did not augment in these tissues (Table I). This is consistent with the observation in the deficiency experiment for 6 weeks.[25]

TABLE I Levels of α-tocopherol, lipid hydroperoxides, TBARS, glutathione, glutathione peroxidase and SOD activities in the vitamin E-deficient rats for 4 weeks and the control animals

		Brain	Heart	Lung	Liver	Kidney	Muscle
α-Tocopherol	Deficient	6.34**	1.19**	0.95**	0.90**	0.55**	1.62**
(μg/g tissue)		±1.52	±0.41	±0.35	±0.23	±0.20	±0.26
	Control	11.47	12.92	15.26	35.69	6.68	8.19
		±2.69	±2.67	±5.41	±12.90	±2.05	±1.43
Lipid	Deficient	184.7	389.2**	345.0*	276.4	300.0*	100.2
hydroperoxide		±41.2	±69.2	±47.4	±95.0	±89.6	±55.0
(pmol/mg proptein)	Control	170.3	217.4	178.5	317.2	168.4	89.1
		±69.5	±89.1	±133.9	±161.1	±56.8	±29.7
TBARS	Deficient	35.3	34.6	39.2	26.8	56.9	13.6*
(nmol MDA/g tissue)		±1.4	±6.5	±10.4	±3.2	±7.1	±1.8
	Control	37.8	28.6	45.6	25.8	50.7	10.6
		±5.6	±3.5	±7.6	±3.5	±8.3	±1.9
Total GSH	Deficient	1.80	1.64	1.87	5.80	2.51	0.88
(μmol/g tissue)		±0.08	±0.10	±0.09	±0.57	±0.17	±0.02
	Control	1.75	1.62	1.54	5.62	2.08	0.90
		±0.07	±0.14	±0.20	±0.35	±0.24	±0.11
Glutathione	Deficient	18.3	137.9	120.4	255.6	215.1	18.4
peroxidase		±7.8	±32.1	±19.4	±23.4	±16.1	±7.6
(nmol NADPH/	Control	17.1	124.7	128.9	262.6	202.2	11.4
mg protein/min)		±11.9	±19.3	±24.5	±39.7	±27.3	±9.7
SOD	Deficient	2.95	3.94	2.62	17.21	8.89	1.15
(NU/mg protein)		±0.78	±0.77	±0.67	±4.12	±1.63	±0.34
	Control	2.27	3.31	2.38	15.60	7.50	0.93
		±0.65	±0.51	±0.52	±2.86	±1.42	±0.15

Rats were fed with vitamin E-deficient diet and the control group was supplied with vitamin E-contained diet. After 4 weeks, levels of α-tocopherol, lipid hydroperoxides, TBARS, glutathione, glutathione peroxidase and SOD activities were determined for the brain, heart, liver, lung and kidney as described in the text. Values are means ± SD of 5 or 6 rats. Asterisks indicate significant difference from the corresponding control (Welch's *t*-test, * $P < 0.05$, and ** $P < 0.01$).

In the brain, hydroperoxide did not augment and the decrease of vitamin E was the slowest among tissues studied in this work. This is consistent with the report[23, 24, 27] that the brain is protected against vitamin E loss compared with other tissues. Recently we reported that the decrease of vitamin C in the brain during ascorbate deficiency in the inherently scorbutic animal (ODS rat) was the slowest among tissues.[28] These results suggested that the brain had some mechanism to retain antioxidant vitamins.

Hydroperoxides in the muscle (femoral) did not increase, while TBARS increased significantly compared with the control animals (Table I). This result demonstrates that lipid peroxidation in the muscle increases to produce TBARS (i.e., aldehydes) but hydroperoxides do not accumulate significantly in a relatively short period (4 weeks) of the vitamin E-deficiency.

Other indicators of radical reactions were also measured and the results were included in Table I. Activities of glutathione peroxidase and SOD were not affected by the deficiency of vitamin E in consonance with the reports,[24, 29] the latter of which described that GSH in the liver increased on more prolonged deficiency of the vitamin. We did not observe any significant difference in the level of GSH as shown in Table I. This result indicates that the vitamin deficiency for 4 weeks was not so severe as the reported case.[30]

Awad et al.[30] reported that F_2-isoprostanes increased in the animals deficient in both vitamin E and selenium for more than 12 weeks and argued that the molecules were proved to be an index of lipid peroxidation. As shown above, the increase of lipid hydroperoxides was detected at only 4 weeks after the start of vitamin E deficiency. Therefore, this index may be the most sensitive among indicators of lipid peroxidation caused by the deficiency of vitamin E.

Vitamin E-deficiency for 8 weeks

The content of α-tocopherol in the brain, heart, lung, liver, kidney and muscle of the deficient animals which were fed with vitamin E-deficient diet for 8 weeks, were all significantly lower than the control as shown in Table II.

The hydroperoxide levels in the heart, lung and kidney of the deficient animals were also significantly higher than those in the corresponding organ of the control rats as shown in Table II. The level of lipid hydroperoxide in other tissues did not increase in the prolonged vitamin E deficiency for 8 weeks. TBARS augmented in the liver, kidney and muscle as compared to the control (Table II). This result demonstrated that the oxidative stress in the liver made vitamin E deficient for 8 weeks increased to produce TBARS, i.e., aldehydes, although accumulation of lipid hydroperoxides was not observed. This observation suggested that the liver had enough capacity to metabolize hydroperoxides into aldehydes which were assumed to be less reactive than peroxide. Lipid hydroperoxide and/or TBARS increased in all tissues except the brain during vitamin E deficiency for 8 weeks. Therefore, these two indices may be useful for the detection of oxidative stress caused by the deficiency of the vitamin, although the relationship between these parameters in each tissue remains to be explored.

In the brain, neither hydroperoxide nor TBARS augmented. This may be explained on the ground that the decrease of vitamin E was the slowest among tissues studied in this work and about a half of vitamin E remained in the brain after 8 weeks of deficiency. The level of GSH and activities of glutathione peroxidase and SOD in all tissues studied in the present experiment were not affected by the deficiency of vitamin E for 8 weeks as shown in Table II. These observations were consistent with the literarure.[24]

Acknowledgements

This work was supported by the Ministry of Science, Education and Culture of Japan.

TABLE II Levels of α-tocopherol, lipid hydroperoxides, TBARS, glutathione, glutathione peroxidase and SOD activities in the vitamin E-deficient rats for 8 weeks and the control animals

		Brain	Heart	Lung	Liver	Kidney	Muscle
α-Tocopherol	Deficient	5.79**	0.367**	0.347**	0.328**	0.222**	0.505**
(μg/g tissue)		±0.38	±0.07	±0.032	±0.068	±0.064	±0.111
	Control	11.5	10.6	12.5	33.5	4.85	8.18
		±1.31	±0.963	±3.77	±6.74	±0.701	±1.03
Lipid	Deficient	187.4	756.8*	483.1**	349.1	449.7**	130.5
hydroperoxide		±39.4	±101.1	±121.1	±119.3	±64.7	±38.6
(pmol/mg proptein)	Control	187.1	520.8	156.0	438.4	311.0	81.0
		±32.4	±169.4	±64.9	±94.0	±34.1	±32.6
TBARS	Deficient	38.6	40.9	30.6	31.5*	68.0*	14.8**
(nmol MDA/g tissue)		±5.24	±5.50	±1.34	±4.07	±6.83	±2.11
	Control	36.1	36.2	39.0	27.2	57.8	8.95
		±2.06	±4.62	±11.2	±1.79	±2.97	±0.96
Total GSH	Deficient	1.80	1.67	1.65	6.16	2.39	0.76
(μmol/g tissue)		±0.08	±0.17	±0.16	±0.41	±0.14	±0.05
	Control	1.63	1.64	1.77	6.64	2.11	0.73
		±0.19	±0.13	±0.03	±0.48	±0.24	±0.08
Glutathione	Deficient	21.3	118.5	139.2	324.8	238.7	18.4
peroxidase		±9.4	±9.8	±9.7	±32.1	±15.1	±7.8
(nmol NADPH/	Control	26.7	123.4	159.4	324.7	231.8	22.1
mg protein/min)		±5.3	±19.4	±22.1	±58.1	±24.1	±5.7
SOD	Deficient	3.35	4.25	3.22	18.05	9.32	1.18
(NU/mg protein)		±0.37	±0.79	±0.55	±1.97	±1.60	±0.25
	Control	2.97	4.37	2.48	15.99	8.45	1.41
		±0.55	±1.07	±0.72	±4.35	±1.73	±0.36

Rats were fed with vitamin E-deficient diet and the control group was supplied with vitamin E-contained diet. After 8 weeks, levels of α-tocopherol, lipid hydroperoxides, TBARS, glutathione, glutathione peroxidase and SOD activities were determined for the brain, heart, liver, lung and kidney as described in the text. Values are means ± SD of 5 or 6 rats. Asterisks indicate significant difference from the corresponding control (Welch's *t*-test, * $P < 0.05$, and ** $P < 0.01$).

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