Restorative Effect of Vitamin E Supplementation on Hepatic Lipid Peroxidation and Lipid Profile Changes Induced by Sublethal γ-Radiation in BALB/c Mice

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ABSTRACT

In order to find a prophylactic supplementation for individuals who are at risk of exposure to ionizing radiation, we attempted to evaluate the effect of Vitamin E (Vit-E), a biological free radical scavenger, on restoration of hepatic lipid peroxidation (LPO) and lipid profile (LP) changes induced by sublethal γ- radiation in BALB/c mice. The concentrations of cholesterol and phospholipid were determined in control and irradiated mice. Also, changes in lipid peroxidation and lipid profile were assessed by measuring the level of malonyldialdehyde, lipid hydroperoxides, and conjugated dienes. Our results showed that sublethal γ-radiation caused significant changes in hepatic lipid peroxidation and lipid profile. However, Vit-E supplementation was able to restore the changes of lipid peroxidation and lipid profile in irradiated mice. We have concluded that the mice that received Vit-E supplementation were able to tolerate biomembrane damage provoked by 1.09 Gy for 3 days γ-radiation. This supports the hypothesis that Vit-E may afford an efficient protection against ionizing radiation. However additional studies using higher doses of γ-irradiation should be performed. *Iran. Biomed. J. 6 (1): 37-41 2002*

Keywords: γ-irradiation, BALB/c, Lipid profile, Lipid peroxidation, Vit-E supplementation

INTRODUCTION

Free radicals can be generated as by-products of the normal cellular redox processes or via the interaction of cells and tissues with a variety of external agents and processes (e.g. thermal or photochemical reactions, ionizing radiation or the action of xenobiotics) [1].

It has been documented that the effect of oxygen-radiosensitizing lethal damage in the cell resides in membrane sites and the influence of oxygen on radiation damage in DNA is less important [2].

The alteration of the functions and properties of membranes can be detected after exposure to relatively low dose of radiation [2]. The two main molecular components of the membrane are lipids and proteins. As lipids are prone to oxidation of unsaturated bonds, it is perhaps reasonable to advocate lipid peroxidation as a significant event in the development of membrane damage [3]. Lipid peroxidation is a complicated radical chain reaction leading to the formation of various products including lipid hydroperoxides, conjugated dienes and malondialdehyde (MDA) [2, 4]. Several methods have been developed to study this radical chain reaction [5, 6]. Detection of lipid hydroperoxides and conjugated dienes and tribarbituric acid-reactive substances (TBARS) such as MDA, often applies to the study of lipid peroxidation reactions [5, 7].

There are several defense mechanisms that protect living organisms against free radicals. Vit-E, a fat-soluble vitamin, is one of the biological antioxidants that protect cell membranes from oxidative damage [4].

Vit-E is present in the blood as dl-alpha-tocopherol acting as the major antioxidant in cell membrane. Vit-E is believed to be the first line of defense against cell membrane damage due to peroxidation. It scavenges free

radicals, terminating chain reactions and confining damage to limited area of the membrane [4].

The prophylactic effect of Vit-E against free-radical damage to cell membrane due to ionizing radiation was evaluated in individuals who are at risk of the exposure. In this study, we made attempts to show the probable mechanism involved in the protective effect of Vit-E in cell membrane damage due to γ-irradiation BALB/c mice were subjected to γ-radiation with or without Vit-E supplementation and LPO level was assessed by determining hepatic lipid fluorescence, conjugated dienes and malondialdehyde levels.

MATERIALS AND METHODS

Animals and diets. Five female BALB/c mice (16-18g, 6-8 week old) (Razi Inst. of Iran, Tehran, Iran) per group were maintained on a regular mice chow in transparent plastic boxes with chip bedding and a stainless steel wire lid for a week. The room temperature was kept at 20-22°C with a constant humidity and a 12:12 h light-dark cycle. Following this adjustment period, mice were divided into two major groups maintained on Vit-E (1 g/kg diet) or regular chow diets [8]. Vit-E was added to the mice chow 3 weeks before γ-irradiation.

Ionizing radiation. Whole body radiation was performed using ^{60}Co-γ-rays from a Gamma cell 220 Machine with a dose-rate of 0.5 Gy/s. Mice receiving 3 Gy (1 Gy/day) did not die, however, their lipid profile and lipid peroxidation significantly changed. Therefore the same dose was selected for Vit-E supplementation study.

Extraction and determination of liver lipids. At the end of exposure, the mice were bled and killed by cervical dislocation. Approximately 0.5 g of the liver was homogenized in 10ml of a 2:1 chloroform/ methanol (v/v) solution (Folch reagent containing 0.05% butylated hydroxytoluene as an antioxidant to prevent *in vitro* peroxidation of lipids) and the homogenates were allowed to stay in sealed vials for 16-18 h at 4°C.

Table 1. Effect of moderate prolonged γ-radiation on hepatic cholesterol and phospholipid in BALB/c mice.

Duration (day)	γ-radiation Dose (Gy)	Cholesterol (mg/g Liver)	Phospholipid (mg/g Liver)	Cholesterol/phospholipid ratios
1	0.00	5.25 ± 0.45	30.29 ± 2.79	0.173
	1.09	8.14 ± 1.83	31.40 ± 3.66	0.126
	1.58	8.70 ± 1.09	30.65 ± 2.44	0.284
	2.30	9.76 ± 0.50*	30.73 ± 2.88	0.320
	3.10	11.72 ± 0.83**	29.15 ± 2.60	0.400
2	0.00	5.99 ± 0.76	32.42 ± 3.26	0.180
	1.09	8.24 ± 0.73*	30.56 ± 3.01	0.270
	1.58	9.85 ± 0.69*	33.62 ± 4.46	0.300
	2.30	10.66 ± 1.41**	31.57 ± 1.62	0.340
	3.10	12.18 ± 1.42**	29.50 ± 4.21	0.412
3	0.00	6.06 ± 0.73	31.30 ± 2.50	0.190
	1.09	9.72 ± 0.55**	30.66 ± 1.58	0.320
	1.58	11.13 ± 0.67**	29.67 ± 2.02	0.370
	2.30	11.27 ± 1.93**	30.09 ± 2.83	0.380
	3.10	14.78 ± 1.85**	28.30 ± 2.61	0.500

*, Significantly different from controls, $P<0.05$; **, Significantly different from controls, $P<0.005$

Table 2. Effect of moderate prolonged γ -radiation on indices of lipid peroxidation in BALB/c mice.

Duration (day)	γ-radiation dose (Gy)	Conjugated Dienes (absorbance unit/g Liver)	Hepatic Fluorescence (unit/g Liver $\times 10^3$)	MDA (nmol/ml serum)
1	0.00	62.4 ± 3.3	10.62 ± 0.99	0.361 ± 0.006
	1.09	73.2 ± 5.5	12.14 ± 1.00	0.382 ± 0.004*
	1.58	77.2 ± 6.2	12.44 ± 0.72*	0.431 ± 0.005**
	2.30	79.8 ± 8.2*	13.86 ± 0.55*	0.473 ± 0.005**
	3.10	82.4 ± 2.4**	13.52 ± 1.00*	0.470 ± 0.007*
2	0.00	59.4 ± 2.4	11.14 ± 1.30	0.349 ± 0.002
	1.09	80.8 ± 1.6	13.12 ± 0.73*	0.420 ± 0.000**
	1.58	86.8 ± 1.9**	13.56 ± 0.56*	0.450 ± 0.007*
	2.30	82.2 ± 3.6*	14.32 ± 0.76*	0.480 ± 0.010**
	3.10	94.8 ± 8.0**	14.18 ± 1.74*	0.586 ± 0.011**
3	0.00	59.4 ± 3.0	10.82 ± 0.49	0.389 ± 0.005
	1.09	80.2 ± 3.1*	13.20 ± 0.71*	0.493 ± 0.005**
	1.58	85.4 ± 3.0**	15.56 ± 0.12**	0.550 ± 0.007**
	2.30	102.0 ± 22.6**	15.48 ± 0.84**	0.840 ± 0.014*
	3.10	109.0 ± 13.0**	15.83 ± 0.45**	1.446 ± 0.005**

*, Significantly different from controls , $P<0.05$; **, Significantly different from controls , $P<0.005$

The phospholipid content of homogenate was determined as described previously [9]. This method of analysis does not require pre-digestion of the phospholipid and is based on using dipalmitoyl phosphatidylcholine as a standard. Total cholesterol was estimated by a method described previously [10].

Determination of hepatic lipid fluorescence and conjugated dienes. The clear Folch homogenate was used to determine hepatic fluorescence as described previously [3]. Fluorescence intensity of the solution was measured at an excitation wavelength of 395 nm and emission wavelength of 435 nm (RF-5000 Spectrophotometer, Shimadzu, Kyoto, Japan). Hepatic dienes conjugated fatty acids were determined as described [11].

Hepatic malondialdehyde determination. Peroxidative damage to the liver was also measured by the formation of malondialdehyde (MDA) using the thiobarbituric acid method described previously [12], using MDA-bis-dimethyl acetal (Aldrich Chem., Milwaukee, Wi) as a standard. Briefly, 0.2 ml of 7% SDS, 0.2 ml of 0.1N HCl, 0.2 ml of 10% phosphotungstic acid and 1 ml of 0.67% tribarbituric acid aqueous solution were added to the liver homogenate. The samples were immediately
heated at 95°C for 60 min. After cooling, the chromogen was extracted with 5 ml of n-butylalcohol by shaking vigorously. The organic phase was separated by centrifugation at 4500 × g for 10min . Fluorescence intensity of the organic phase was measured at excitation wavelength of 515 nm and emission wavelength of 553 nm.

Data analysis. A two-way analysis of variance, student's *t*-test and linear regression analysis for correlation coefficient were used in statistical calculations where appropriate. The results are presented as the mean ± S.D. $p<0.05$ was considered significant.

RESULTS

As shown in Table 1, γ-radiation significantly altered the hepatic lipid profile. The level of cholesterol was increased at doses 2.3 and 3.1 Gy after one day exposure compared to control ($p<0.05$). Such increment of cholestrol tended to increase as dose and time of the exposure mounted. Although the level of hepatic phospholipid did not change, the ratio of cholesterol/phospholipid showed a slight augmentation (Table 1).

Table 3. Effect of Vit-E supplementation on hepatic cholesterol and phospholipid in radiated and non radiated BALB/c mice.

γ-radiation[#]	Vitamin E[##] Supplementation	Total cholesterol (mg/g liver)	Phospholipid (mg/g liver)	Cholesterol/Phospholipid ratios
-	-	6.83 ± 0.90	26.92 ± 0.36	0.26
	+	6.44 ± 0.74	25.30 ± 1.23	0.25
+	-	9.20 ± 0.77	28.00 ± 0.87	0.32
	+	7.13 ± 0.63*	26.20 ± 1.16	0.27

#, 109Gy/day for 3days; ##, 1g/Kg/day 3 weeks prior to exposure; *, Significantly different from controls, $P<0.05$.

Table 4. Effect of Vit-E supplementation on indices of lipid peroxidation in radiated and non radiated BALB/c mice.

γ-radiation[#]	Vitamin E Supplementation[##]	Conjugated Dienes (absorbance unit/g Liver)	Hepatic Fluorescence (unit/g Liver $\times 10^3$)	MDA (nmol/ml serum)
-	-	57.61 ± 3.2	11.77 ± 1.06	0.31 ± 0.004
	+	50.60 ± 1.2	10.90 ± 0.38	0.29 ± 0.012
+	-	76.00 ± 12.0	14.34 ± 0.69	0.48 ± 0.008
	+	56.00 ± 2.0*	12.35 ± 1.24*	0.33 ± 0.007**

#, 1.09 Gy/day for 3 days; ##, 1g/kg/day 3 weeks prior to exposure; *, Significantly different from controls, $P<0.05$; **, Significantly different from controls, $P<0.005$

To verify any changes in lipid peroxidation subsequent to exposure to γ-radiation, we measured the concentration of MDA in serum and determined the level of hepatic conjugated dienes and lipid fluorescence (Table 2).

In corroboration of lipid profile results, we observed time and dose increment in hepatic conjugated dienes, lipid fluorescene and serum level of MDA (Table 2). Taken together, we were able to show the effect of γ-radiation on cell membrane damage due to lipid peroxidation even at the lowest dose of 1.09 Gy for up to 3 days, which was subsequently selected to evaluate the restorative effect of Vit-E supplementation on hepatic lipid peroxidation

Table 3 and 4 show the summarized results of Vit-E supplementation. Although Vit-E supplementation did not make any alteration on hepatic lipid profile and lipid peroxidation, it clearly protected mice against deteriorative effect of γ-radiation on cell membrane oxidation.

DISCUSSION

Our results showed that γ-radiation damages cell membrane by altering lipid profile and LPO. These data are in agreement with recent studies demonstrating that free radical attack on hydrophilic moiety, along with lipid peroxidation, may constitute the principal mechanism of radiation-induced damage of biological membranes [13]. It is noteworthy that even chronic low-dose gamma radiation adversely affected the activity of the lipid free-radical oxidation [14,15]. Additionally, γ-radiation damages biological antioxidant systems by means of decreasing the level of Vit-E in membranes [16, 17] and inhibiting superoxide dismutase transcription [18]. In addition, Vit-E deficiency is associated with free radical-induced liver injury. Such conditions may occur for individuals who are at risk of exposure to ionizing radiation [19].

This radioprotective effect of Vit-E has already been shown in the course of epidermal cell damage due to ultraviolet radiation *in vitro* [20]. In parallel we showed that Vit-E supplementation was able to completely confine the membrane damage induced by γ-radiation in BALB/c mice.

In conclusion we showed that: γ-radiation affected cell membrane properties by changing LP and LPO even at relatively low doses, 1.09 GY for up to 3 days. Vit-E supplementation was able to restore this deteriorative effect.

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