EFFECT OF CIMETIDINE AND RANITIDINE ON LIPID PROFILE AND LIPID PEROXIDATION IN γ-IRRADIATED MICE

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Abstract- Elevated amounts of free radicals due to ionizing radiation have damaging effects on the body. H2-receptor antagonists have potential oxygen radical scavenging properties. We tried to determine the effects of two H2-receptor antagonists (cimetidine and ranitidine) on lipid peroxidation (LPO) and lipid profile (LP) in plasma and liver of γ-irradiated (1Gy/day for 3 days) BALB/c mice. The control group of mice were fed with normal food and drinks but the experiment group of mice were fed control diet and drinking water, containing cimetidine or ranitidine (1mg/lit). After 3 days of supplementation, the animals were subjected to sublethal γ-radiation, which caused a significant increase in cholesterol level in experimental group (100% increase in comparison with the control group), but the amount of phospholipids did not change. The ratio of cholesterol to phospholipid showed a slight increase. Also γ-irradiation caused a significant increase in lipid fluorescence (11-48%), conjugated dienes (33-81%) in liver and increase in malondialdehyde (19-300%) in serum of mice who had received 1.09-3.1 Gy for 1-3 days. Cimetidine or ranitidine supplementation was able to restore the changes of LPO and LP in mice (1 Gy-radiated for 3 days). It is therefore concluded that the mice treated with cimetidine or ranitidine were able to tolerate biomembrane damages provoked by sublethal γ-radiation. This supports the hypothesis that cimetidine or ranitidine may afford an efficient protection against ionizing radiation or diseases that are characterized by *in vivo* free radical-mediated oxidative stress mechanisms.

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INTRODUCTION

Biological materials are able to absorb energies of radiation by inducing ionization or reactivation. Radiation can cause production of free radicals, which can cause metabolic disturbances and cell injury in a variety of ways (Fig. 1) (1).

Hydroxyl radicals are highly reactive but their average radius of diffusion is small and their half-life in biological systems is only a few microseconds (2). For these reasons, free radicals, especially hydroxyl radical (OH°) can induce peroxidation of polyunsaturated fatty acids in the cell membrane where free radical intermediates are produced in excess. There is increasing evidence that in certain circumstances protective systems may be overwhelmed, and cell damage by oxygen radicals and lipid peroxidation plays a crucial and causative role in the pathogenesis of several acute and chronic diseases such as cancer, inflammation, shock, liver injury, rheumatoid arthritis, aging and atherosclerosis (3). H2-receptor antagonists (cimetidine and ranitidine) have potential oxygen radical scavenging properties. They can scavenge OH° with rate constant 1.6×10^{10} $mol^{-1}s^{-1}$ and 7.5×10^{9} mol^{-1} s^{-1} for cimetidine and ranitidine, respectively (4). In this study the preventive effect of cimetidine and ranitidine against free radical damages to cells is reported. BALB/c mice were exposed to γ-radiation with or without cimetidine or ranitidine supplementation and their lipid peroxi-dation (LPO) and lipid profile (LP) levels were assessed.

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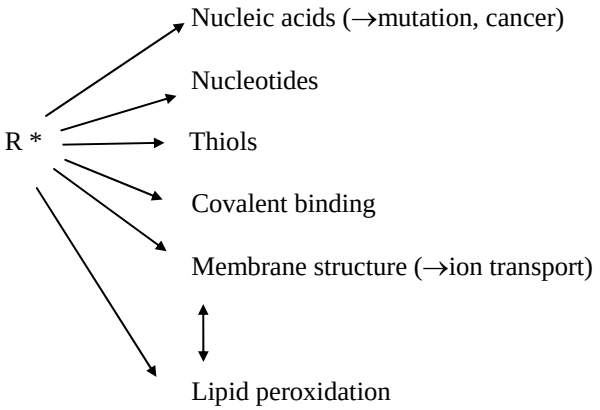

Fig. 1. Free radicals and cellular injury

MATERIALS AND METHODS

Animals and Treatments

Female BALB/c mice, 6-8 week old, were obtained from the Razi research institute. of Iran (Hesarak, Karaj). The mice were housed, five animals per cage, in transparent plastic boxes with chip bedding and a stainless steel wire lid in the animal facility at Tehran University. The room temperature was kept at 20-22°C under constant humidity and a 12 h light-dark cycle. All experiments on animals were performed in accordance with UK legal requirements. Following a period of 2 weeks adaptation, mice were randomly divided into 4 groups, 5 mice in each group, and were subjected to 0, 1.09, 2.03 and 3.02 Gy/day γ-radiation respectively. In the second experiment the mice were assigned to one of the following three categories: the control group which drunk tap water only but the other groups received either cimetidine (1mg/lit) or ranitidine (1mg/lit) for three days in tap water.

Ionizing Radiation

Irradiation with ionizing radiation was performed using ^{60}Co-γ-rays from a Gamma cell 220 machine with a dose-rate of 0.1 Gy/s.

The dose of radiation varied from 0-3 Gy/day for 1-3 days at maximum 9 Gy/3 days. Mice who were exposed to 1Gy/day radiation for 3 days, 3 Gy in total, did not die, but their LP and LPO levels significantly changed. Therefore, the dose of 1 Gy/day for 3 days was chosen for the group that received cimetidine or ranitidine treatment. At the end of exposure the blood was collected, mice were killed by dislocation and the livers were collected and kept at -20°C until used.

Extraction and Determination of Liver Lipids

Approximately 0.5g of liver was homogenized in 10 ml of a 2:1 (v/v) mixture of chloroform, methanol (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.

Phospholipid content of the homogenate was determined as previously described (5). 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 the method previously described (6).

Determination of Hepatic Lipid Fluorescence and Conjugated Dienes

The clear Folch homogenate was used to determine the hepatic fluorescence as previously described (7). Fluorescence intensity of the solution was measured at the excitation wavelength of 395nm and emission wavelength of 435nm (RF-5000 Spectrofluorimeter, Shimadzu, Kyoto, Japan). Hepatic diene conjugated fatty acids were determined as previously described (8).

Serum Malondialdehyde Determination

Peroxidative damage was also measured by the formation of malondialdehyde (MDA) using the thiobarbituric(TBA) acid method previously described (9) using MDA-bis-dimethyl acetal (Aldrich Chem., Milwaukee, Wi) as a standard. Briefly, 0.2ml of 7% sodium dodecyl-sulfate (SDS), 0.2ml of 0.1N HCl, 0.2ml of 10% phosphotungstic acid and 1 ml of 0.67% TBA aqueous solution were added to serum.

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 3000 rpm for 10 min. Fluorescence

intensity of the organic phase was measured at the excitation wavelength of 515 nm and emission wavelength of 553 nm.

Data analysis

Two way analysis of variance, *t* test and linear regression analysis for correlation coefficient were used in statistical calculations. The results were presented as the mean ± SD; *P* values <0.05 were 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 of exposure compared to control ($P<0.05$) and tended to increase as dose and time of exposure were increased. The mean concentration of cholesterol of the control mice was 5.25 mg/g of liver. However the concentration of cholesterol in the liver of mice receiving 2.3 and 3.1 Gy γ– irradiation showed about two-fold increment (9.76 and 11.72 mg/g of liver, respectively).

In contrast, the levels of phospholipids (mg/g liver) did not show any significant difference. The mean level of phospholipids in the liver of control mice was 31 mg/g of liver; the same level was obtained even in the liver of mice receiving 3.1 Gy γ– irradiation for 3 days, which was the highest dose in the study (Table 1).

Although the level of hepatic phospholipids did not change, the ratio of cholesterol/phospholipid showed a slight augmentation (Table 1).

To verify any change in lipid peroxidation subsequent to exposure, the concentration of MDA in serum was measured and the levels of hepatic conjugated dienes and lipid fluorescence were determined (Table 2). The mean level of conjugated dienes of control mice was 60 unit/g of liver. However, the mice exposed to even 2.3 Gy and 3.1 Gy for 1 day showed a significant increase in conjugated dienes, which were 79.8 and 82 units/g liver respectively. This increment was observed for hepatic fluorescence and serum levels of MDA.

The mean level of hepatic fluorescence of the control mice was about 10900 unit/g of liver but even low level of irradiation increased it to 13520 (Table 2). In parallel, MDA mean level of control mice were about 0.38 nmol/ml serum in contrast to the γ– irradiate mice, which showed significant increase in serum MDA (Table 2).

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

Duration (day)	γ-radiation dose (Gy)	Cholesterol (mg/g of liver)	Phospholipid (mg/g of liver)	Cholesterol/phospholipid ratio
1	0	5.25±0.425	30.29±2.79	0.173
	1.09	8.14±1.830	31.4±3.66	0.126
	1.58	8.70±1.09	30.65±2.44	0.284
	2.30	9.76±0.5*	30.73±2.88	0.320
	3.10	11.72±0.83†	29.15±2.60	0.400
2	0	5.90±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†	29.5±4.21	0.412
3	0	6.06±0.73	31.3±2.5	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†	28.3±2.61	0.500

* Significantly different from controls, *P* value<0.05.

† Significantly different from controls, *P* value<0.005.

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

Duration (day)	γ-radiation dose (Gy)	Conjugated dienes (unit/g of liver)	Hepatic Fluorescence (unit/g of liver)	MDA (nmol/ml serum)
1	0	62.4±3.3	10620 ± 990	0.361±0.006
	1.09	73.2±5.5	12140±1000	0.382±0.002*
	1.58	77.2±6.2	12440 ± 720*	0.431±0.005†
	2.30	79.8±8.2*	13860± 550*	0.473±0.005†
	3.10	82.4±2.4†	13520±1000*	0.470±0.005*
2	0	59.4±2.4	11140±1300	0.349±0.002
	1.09	80.8±1.6	13120±730*	0.420±0.000†
	1.58	86.8±1.96†	13560±560*	0.450±0.007*
	2.30	82.2±3.6*	14320±760*	0.480±0.010†
	3.10	94.8±8.0†	14180±1740*	0.586±0.011†
3	0	59.4±3	10820±490	0.389±0.005
	1.09	80.2±3.1*	13200±710*	0.493±0.005†
	1.58	85.4±3†	15560±120†	0.550±0.007†
	2.30	102±22.6†	15480±840†	0.840±0.014*
	3.10	109±13†	15830±450†	1.446±0.005†

* Significantly different from controls, *P* value<0.05.

† Significantly different from controls, *P* value<0.005.

In corroboration with the lipid profile results, a time and dose increment hepatic conjugated dienes, lipid fluorescence and serum level of MDA were observed (Table 2).

Through these findings, we were able to show that the effects of γ-radiation on cells is due to lipid peroxidation even at the lowest dose of radiation (1.09 Gy for up to 3 days), which was subsequently selected as our experimental dose, to evaluate the restorative effect of cimetidine or ranitidine supplementation on hepatic lipid peroxidation.

Tables 3 to 6 summarize the effects of cimetidine and ranitidine supplementation. Table 3 shows the effect of cimetidine supplementation on hepatic cholesterol and phospholipid of irradiated and non–irradiated mice. The total level of hepatic cholesterol of control mice was 6.8 mg/g of liver and for mice receiving only cimetidine was 5.9 mg/g of liver, which was not significantly different from the control mice. However, cimetidine supplementation was able to reduce the hepatic cholesterol with no significant difference between the exposed mice receiving cimetidine and the control mice (Table 3). Again no change in phospholipid was observed in any of the experimental groups of mice. Table 4 shows the effect of ranitidine supplementation on hepatic cholesterol and phospholipid in exposed and unexposed mice. Similar to cimetidine, ranitidine inhibited the increment of hepatic cholesterol in the exposed mice. Tables 5 and 6 show the results of lipid peroxidation indices of exposed and unexposed mice. There was no change in the levels of conjugated dienes, hepatic fluorescence, or serum MDA in supplemented mice. However, irradiated mice receiving supplementation showed a significant decreased of lipid peroxidation indices (Tables 5 and 6).

Although cimetidine and ranitidine supplementation did not alter the hepatic lipid profile and lipid peroxidation on their own, they clearly protected the mice against the damaging effects of γ-radiation due to lipid peroxidation.

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

γ-radiation*	Cimetidine supplementation†	Total cholesterol (mg/g liver)	Phospholipid (mg/g liver)	Cholesterol/phospholipid ratios
-	-	6.83±0.9	26.92±0.36	0.26
	+	5.9 ±0.79	26.06 ±1.08	0.23
+	-	9.2±0.77	28±0.87	0.32
	+	6.72±0.27‡	26±2.21	0.26

* 1.09 Gy/day for 3days.

† 1mg/lit/day 3 days prior to exposure.

‡ Significantly different from controls, *P* value<0.05.

Table 4. Effect of ranitidine supplementation on hepatic cholesterol and phospholipid in radiated and non-radiated BALB/c mice

γ-radiation*	Ranitidine supplementation†	Total cholesterol (mg/g wet tissue)	Phospholipid (mg/g wet tissue)	Cholesterol/phospholipid ratios
-	-	6.83±0.9	26.92±0.36	0.26
	+	6.93 ±0.26	27.53 ±2.53	0.25
+	-	9.2±0.77	28±0.87	0.32
	+	6.68±1.34‡	27.3±0.8	0.25

* 1.09 Gy/day for 3 days.

† 1mg/lit/day 3 days prior to exposure.

‡ Significantly different from controls, *P* value<0.05.

Table 5. Effect of cimetidine supplementation on indices of lipid peroxidation in radiated and non-radiated BALB/c mice

γ-radiation*	Cimetidine supplementation†	Conjugated dienes (unit/g liver)	Hepatic fluorescence (unit/g liver)	MDA (nmol/ml serum)
-	-	57.61±3.2	11770±1056	0.316±0.008
	+	64±2	11434±954	0.254±0.011
+	-	76±12	14344±688	0.482±0.017
	+	66±4‡	12132±470*	0.342±0.032

* 1.09 Gy/day for 3days.

† 1mg/lit/day 3 days prior to exposure.

‡ Significantly different from controls, *P* value<0.05.

Table 6. Effect of ranitidine supplementation on indices of lipid peroxidation in radiated and non-radiated BALB/c mice

γ-radiation*	Ranitidine supplementation†	Conjugated dienes (unit/g liver)	Hepatic fluorescence (unit/g liver)	MDA (nmol/ml serum)
-	-	57.61±3.2	11770±1056	0.316±0.008
	+	58±2	11422±524	0.230±0.022
+	-	76±12	14344±688	0.482±0.017
	+	64±6‡	11100±608§	0.346±0.013§

* 1.09 Gy/day for 3days.

† 1mg/lit/day 3 days prior to exposure.

‡ Significantly different from controls, *P* value<0.05.

§ Significantly different from controls, *P* value<0.005.

DISCUSSION

The results showed that γ-radiation changes the lipid profile and increases lipid peroxidation. These findings are in agreement with those reported by others, regarding the effects of oxidants like Fe^{+3}(10), Cu^{++} (1) and ethanol (11), showing a significant increase in the cholesterol levels without affecting PL levels. Since the cholesteryl in low-density lipoprotein is converted to cholesterol upon oxidation, cholesterol/phospholipid ratio was seen to increase. This along with the increase in lipid peroxidation was shown to be the principal damage induced by radiation in biological membranes (12).

Additionally, γ-radiation damages biological antioxidant systems by means of decreasing the level of vitamin E in membranes (12,13) and inhibiting superoxide dismutase transcription (14). Such conditions may occur in individuals who are at risk of exposure to ionizing radiation. In this study, it was seen that cimetidine and ranitidine could control these changes; therefore they can be used as radioprotective drugs. H2-receptor antagonists are scavengers of hydroxyl radicals with a very high rate constant (15). They can stimulate superoxide dismutase activity and decrease MDA concentration in blood platelets of patient with peptic ulcer disease (16). The antioxidant effects of H2-receptor antagonists, such as cimetidine, ranitidine, famotidine (16), Zantac (17) and LT-066 (13), have already been reported in the blood of patients with peptic ulcer and in the gastric mucosa after ischemia-reperfusion. The radioprotective effect of cimetidine has been shown *in vitro* (18) and *in vivo* (19) using micronucleus test. This study provides further evidence that ranitidine and cimetidine can act as *in vivo* radioprotective agents. In addition to the previous mechanisms introduced by other studies, we propose the radioprotective effect of H2-receptor antagonists on lipid profile and lipid peroxidation.

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