The effect of magnesium and vitamin E pre-treatments on irradiation-induced oxidative injury of cardiac and pulmonary tissues in rats: a randomized experimental study

Profilaktik magnezyum ve E vitamini uygulamasının sıçanlarda ışınlamaya bağlı kalp ve akciğer dokusunda gelişen oksidan hasara etkileri: Randomize deneysel bir çalışma

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ABSTRACT

Objective: The aim of this study was to investigate the effect of pre-treatment with the free radical scavenging molecules, magnesium and vitamin E, on lipid peroxidation to limit radiation-induced heart and lung injury.

Methods: Female Sprague-Dawley rats were divided into 4 groups by a simple randomization method as saline-treated control (n=4), saline-treated irradiated (IR; n=6), magnesium sulphate-treated irradiation (IR) (Mg+IR; n=6) and vitamin E-treated IR (vit E+IR; n=6), respectively. The animals were given either saline, Mg (600mg/kg/day) or vit E (100 mg/kg/day) intraperitoneally for five days prior to irradiation. Twelve hours after the fifth injection, animals in irradiation groups were irradiated to 20 Gy using 6 MV photons in linear accelerator. Twenty-four hours later cardiac and lung tissue samples were obtained for determination of myeloperoxidase activity (MPO), malondialdehyde (MDA) levels, and luminol and lucigenin levels measured by chemiluminescence (CL) methods.

Results: No significant changes were observed between cardiac and pulmonary MDA and CL results of the experimental groups. However, cardiac and pulmonary MPO activities in the saline-treated IR group were increased as compared to control group ($p<0.05$ for all), while in the Mg-pretreated and vit E pretreated groups neutrophil infiltration was reduced, reaching to statistical significance only in the Mg-pretreated group ($p<0.05$).

Conclusion: Prophylactic use of magnesium sulfate has limited the infiltration of neutrophils to both the cardiac and pulmonary tissues at the early 24 h of irradiation. However, how limiting neutrophils as the sources of free radicals and inflammatory mediators would alter oxidative stress of heart and lung tissues in the long-term is not clear yet. *(Anadolu Kardiyol Derg 2012; 12: 508-14)*

Key words: Heart, irradiation, lung, magnesium, radioprotection, vitamin E

ÖZET

Amaç: Serbest radikal süpürücü moleküller olan magnezyum sülfat ve E vitamininin profilaktik kullanımının radyasyonun tetiklediği kalp ve akciğer hasarının önlenmesinde lipit peroksidasyonu üzerindeki etkileri incelendi.

Yöntemler: Sprague-Dawley türü dişi sıçanlar basit rastgele randomizasyon yöntemi kullanılarak fizyolojik tuzlu su uygulanan kontrol (n=4), tek başına radyasyon (IR; n=6), radyasyon+magnezyum (Mg+IR; n=6) ve radyasyon+vitamin E (vit E+IR; n=6) ön-tedavisine göre gruplandırıldı. Fizyolojik tuzlu su, Mg (600 mg/kg/gün) ya da vit E (100 mg/kg/gün) radyasyondan önce 5 gün boyunca intraperitonal olarak uygulandı. Son enjeksiyondan 20 saat sonra radyasyon uygulanacak tüm gruplardaki sıçanlara 6 MV foton enerjisi ile 20 Gy iyonizan radyasyon uygulandı. Yirmi-dört saat sonra alınan kalp ve akciğer dokularında miyeloperoksidaz aktivitesi (MPO), malondiyaldehid (MDA) ve kemolüminesans (KL) yöntemi ile lüminol ve lusigenin düzeylerine bakıldı.

Bulgular: Deney grupları arasında kalp ve akciğer dokularının MDA ve KL sonuçları arasında anlamlı bir fark bulunamadı. Ancak, fizyolojik tuzlu su tedavili ışınlama (IR) grubunun kalp ve akciğer MPO aktiviteleri kontrol grubuna göre yüksek bulunurken (hepsi için $p<0.05$), Mg-ön tedavili ve

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vitE-ön tedavili IR gruplarında nötrofil infiltrasyonunun azaldığı gözlendi; fakat bu azalma sadece Mg-ön tedavili grupta istatistiksel olarak anlamlı düzeye ulaştı ($p<0.05$).
Sonuç: Magnezyum sülfatın profilaktik kullanımı ışınlamanın erken 24. saatinde kalp ve akciğer dokusuna nötrofil göçünü sınırlandırmıştır. Ancak, serbest radikallerin ve enflamatuvar mediyatörlerin kaynağı olan nötrofilleri sınırlamanın akciğer ve kalp dokularında ışınlamaya bağlı uzun dönemde gelişecek oksidan hasara nasıl etki edeceği henüz açıklanamamıştır. *(Anadolu Kardiyol Derg 2012; 12: 508-14)*
Anahtar kelimeler: Akciğer, irradyasyon, kalp, magnezyum, radyoproteksiyon, vitamin E

Introduction

In routine clinical practice, administering a curative dose of ionizing radiation to the tumor leads to normal tissue side effects and this may restrict the delivery of high doses of radiation to malignant tissues. Moreover, during the irradiation (IR) and early post-irradiation period, there is a clinical silent period when irradiation damage is initiated, which may then lead to enhanced late damages (1). Tumors located in the chest cavity, such as breast cancer and lymphoma, can be treated with radiotherapy (RT), while the heart, either totally or partially, is within the irradiation field. Although in these irradiated patients the expected overall survival has prolonged, long-term cardiac diseases may occur (2-4). Using new planning techniques or more effective radioprotectors in RT was found to be partially beneficial in decreasing the injury of normal tissues in the treatment fields (5). Heart, as a late responding tissue, deserves high attention in RT (6-8). Therefore, one may say that, in radiation-induced heart disease, the obvious best treatment is to prevent the occurrence of heart disease, if possible.

Lung is mostly known for its high sensitivity to deleterious effects of radiation (9). The sequential and/or concomitant multimodality treatments for thoracic region tumors increase the incidence and severity of lung injury (10). Evidence has shown that the acute inflammatory and late fibrosing stages of radiation-induced injury are not separate pathogenetic entities and the clinical manifestations are part of a pathogenetic process (9, 11).

Besides its direct cytotoxic effect, ionizing radiation also induces a complex inflammatory process in normal tissues. In this study, we investigated the prophylactic effect of magnesium sulfate (Mg) and vitamin E (vit E) as radioprotectors. Magnesium is an N-methyl-D-aspartate receptor antagonist (12) and it may inhibit lipid peroxidation (13, 14). It may indirectly reduce levels of lipid peroxidation by-products, possibly by acting as a glutamate antagonist (15). Vitamin E is a fat-soluble vitamin that acts as an antioxidant, protecting cell membranes. It suppresses lipid peroxidation either by trapping peroxyl radicals involved in the peroxidation chain or by reacting with lipid peroxyl radicals (16). Vitamin E can interfere with the development of radiation-induced fibrosis in the rat lung by acting as an anti-oxidant (17).

However, there are no studies investigating the role of prophylactic Mg administration on irradiated lung and heart tissues and there are only a few studies on the role of vit E (17, 18).

In this study, we aimed to investigate whether these agents interfere with the very early phase of lipid peroxidation by acting as an anti-oxidant and therefore may limit radiation-induced lung and heart injury in rats.

Methods

Study design

This is a randomized controlled experimental study.

Animals

Female Sprague-Dawley rats (250-300 g) supplied by the Marmara University (MU) Animal Center (DEHAMER) were housed in an air-conditioned room with 12-hour light and dark cycles, where the temperature (22±2°C) and relative humidity (65-70%) were kept constant. Rats were fed with standard laboratory chow with free access to water.

A total of 22 rats were divided into 4 groups by a simple randomization method: control (n=4), saline-treated irradiated (IR) (n=6), magnesium sulphate-treated irradiated (IR+Mg) (n=6) and vitamin E-treated irradiated (IR+vit E) (n=6), respectively.

All experimental protocols were approved by the MU School of Medicine Animal Care and Use Committee. All experimental protocols were approved by the Animal Care and Use Committee in our university, which is licensed by the Central Ethical Committee based on Scientific Procedures Act, 1986.

Experimental procedures

The animals were pretreated either with saline, Mg (600mg/kg/day; Biofarma, İstanbul, Turkey) or with vit E (100 mg/kg/day; dl-alpha tocopherol acetate, Aksu Farma, İstanbul, Turkey) intraperitoneally for five days prior to IR.

Irradiation procedure

Irradiation field (from lung apex on top to right below the diaphragm and limited outside the thoracic wall on both sides) covered the whole lungs and heart of the animals. According to linear quadratic model single dose of 20 Gy on the heart is the bioequivalent to the dose of 60 Gy delivered in 30 fractions of 2 Gy (α/β ratio of 3.7 Gy). Krause et al. (19) reported that the dose of 22.5 Gy in single fraction causes death in animals due to cardiac failure. Moreover, single dose of 20 Gy causes total body irradiation (TBI)-induced pneumonitis (20).

Twelve hours after the fifth injection the rats in IR, Mg+IR and vit E+IR groups were irradiated in prone position using a LINAC (Saturne 42, General Electric, France) producing 6 MV photons at a focus-skin distance of 100 cm. Initially, animals were anaesthetized with ketamine (50mg/kg, i.p.) and xylazine (9 mg/kg, i.p.). Following the radiation procedure, where each animal received a single dose of 20 Gy, animals were returned to their home cages. Meanwhile, control group was anaesthetized and immobilized without irradiation.

All animals were sacrificed 24 hours after the irradiation or sham-irradiation. Samples were collected from two different parts of the heart (left and right ventricles) and from four different parts of the lung (left and right apex and base). The tissues were used for the analyses of myeloperoxidase (MPO) and malondialdehyde (MDA), and for the chemiluminescence (CL) assay.

Measurement of MPO activity

Myeloperoxidase is an enzyme that is found predominantly in the azurophilic granules of polymorphonuclear leukocytes (PMN). Tissue MPO activity is frequently utilized to estimate tissue PMN accumulation in inflamed tissues and correlates significantly with the number of PMN determined histochemically in tissues (21). MPO activity was measured in tissues in a procedure similar to that documented by Hillegas et al. (22). Tissue samples were homogenized in 50 mM potassium phosphate buffer (PB, pH 6.0), and centrifuged at 41.400 g (10 min); pellets were suspended in 50 mM PB containing 0.5% hexadecyltrimethylammonium bromide (HETAB). After three freeze and thaw cycles, with sonication between cycles, the samples were centrifuged at 41.400 g for 10 min. Aliquots (0.3 mL) were added to 2.3 ml of reaction mixture containing 50 mM PB, o-dianisidine, and 20 mM H_2O_2 solution. One unit of enzyme activity was defined as the amount of MPO present that caused a change in absorbance measured at 460 nm for 3 min. MPO activity was expressed as U/g tissue.

Measurement of lipid peroxidation

The level of MDA in each tissue specimen was taken to reflect the degree of lipid peroxidation in the tissue. Levels of MDA were determined using the method described by Yagi et al. (23). Briefly, the lung and cardiac tissues were homogenized in ice-cold trichloroacetic acid (TCA, 10% v/v) and then centrifuged. The supernatant was transferred to a test tube containing an equal volume of TCA (0.67% w/v), and this mixture was then heated to 90°C and maintained at that temperature for 15 minutes. The MDA concentration for each specimen was determined in a spectrophotometer based on the level of absorbance at 532 nm, and was expressed as nmol/g tissue.

Chemiluminescence assay

To assess the role of reactive oxygen species in radiation-induced tissue damage, luminol (cytoplasmic injury indicator of oxidative stress) and lucigenin (mitochondrial injury indicator of oxidative stress) chemiluminescences were measured as indicators of radical formation. Measurements were made at room temperature using Junior LB 9509 luminometer (EG&G Berthold, Germany). Specimens were put into vials containing PBS-HEPES buffer (0.5 M PBS containing 20 mM HEPES, pH 7.2). Reactive oxygen species (ROS) were quantitated after the addition of enhancers such as lucigenin or luminol for a final concentration of 0.2 mM. Luminol detects a group of reactive species, i.e. .OH, H_2O_2, HOCl radicals and lucigenin is selective for O^-_2 (24-26). Counts were obtained at 1 minute intervals and the results were given as the area under curve (AUC) for a counting period of 5 minutes. The counts were corrected for wet tissue weights and expressed as relative light units (27).

Statistical analysis

Statistical analyses were performed using SPSS version 10.0 software for Windows (SPSS Inc, Chicago, II, USA). Group data were statistically compared using one-way analysis of variance (ANOVA) followed by Bonferroni multiple comparison test. Student's t-test (unpaired) was used for comparison between two treatment groups. All results were expressed as mean±SEM. The significance level was set at $p<0.05$.

Results

No mortality and no side effects were observed in the experimental groups. There were no clinical signs observed regarding heart and lung injury following irradiation. After the animals were sacrificed, no pericardial or pleural effusions, no macroscopic changes in myocardial or lung tissues were observed. All results are presented in Table 1.

Cardiac tissue results

Twenty-four hours after a single dose of irradiation, no significant changes were observed between MDA and luminol results of the experimental groups, showing no significant changes in the cardiac tissues in the early phase of irradiation (Fig. 1B,C). In lucigenin results, IR group was decreased as com-

Table 1. MPO, MDA and lipid peroxidation results in experimental groups

Variables	Groups			
	Saline	IR	IR+Mg	IR+vit E
Cardiac tissue				
MPO, U/g tissue	17.28±6.41	37.6±5.25*	13.93±4.44**	29.52±6.07
MDA, nmoL/g tissue	17.10±0.89	16.83±0.52	18.56±0.52	16.93±0.31
Luminol, cpm x10^5	43.20±6.46	37.98±5.87	35.60±3.79	40.10±7.94
Lucigenin, cpm x10^5	34.15±3.40	20.68±2.26*	26.91±4.44	21.71±3.77
Lung tissue				
MPO, U/g tissue	20.30±3.07	13.47±2.29¶	6.56±0.29¶¶+	12.82±2.35
MDA, nmoL/g tissue	18.92±1.77	19.65±1.42	17.38±1.47	19.96±2.99
Luminol, cpm x10^5	16.37±2.73	16.51±2.17	15.00±1.95	13.68±1.97
Lucigenin, cpm x10^5	7.70±0.26	15.55±2.57	13.20±2.34***	11.86±1.79

Data are presented as mean±SEM
ANOVA with posthoc Bonferroni multiple comparison test:
Cardiac tissue samples- *$p<0.01$ compared with saline-treated group; **$p<0.05$ compared with IR group
Lung tissue samples - ¶$p<0.05$ compared with saline-treated group; ¶¶$p<0.001$ and ***$p<0.01$ compared with saline-treated group; +$p<0.05$ compared with IR group
CL - chemiluminescence assay, MDA - malondialdehyde, Mg - magnesium sulphate, MPO - myeloperoxidase, vit - vitamin

pared to control group (p<0.01) but neither Mg nor vit E had an effect on reducing the lipid peroxidation (Fig. 1D). However, MPO activity in the saline-treated IR group was increased (37.8±12.4 U/g tissue) as compared to control group (14.7±8.6 U/g tissue; p<0.01), while in the Mg-pretreated (17.1±11.4 U/g tissue; p<0.05) and vit E pretreated (29.52±14.88 U/g tissue; p>0.05) groups neutrophil infiltration was reduced, reaching to statistical significance only in the Mg group (Fig. 1A).

Lung results

As in the cardiac tissue, single dose of irradiation and its short-term effects did not cause any change in pulmonary MDA levels (Fig. 2B). On the other hand, lucigenin but not luminol has detected an enhanced generation of free radicals (p<0.01) in saline-treated IR group, which was not observed in the Mg or vit E-treated IR group (Fig. 2C, D). The pulmonary MPO activity in the saline-treated IR group (13.4±5.6 U/g tissue) was different from the non-irradiated control group (20.3±6.2 U/g tissue; p<0.05) (Fig. 2A). Mg pretreatment before irradiation significantly depressed pulmonary neutrophil infiltration (6.6±0.7 U/g tissue; p<0.001), while vit E pretreatment did not have a significant effect on MPO activity (12.8±5.8 U/g tissue) (p>0.05).

Discussion

Our study shows that neither Mg nor vit E had an effect on reducing the lipid peroxidation on irradiated cardiac or lung tissue. However, only Mg pretreatment before irradiation signifi-

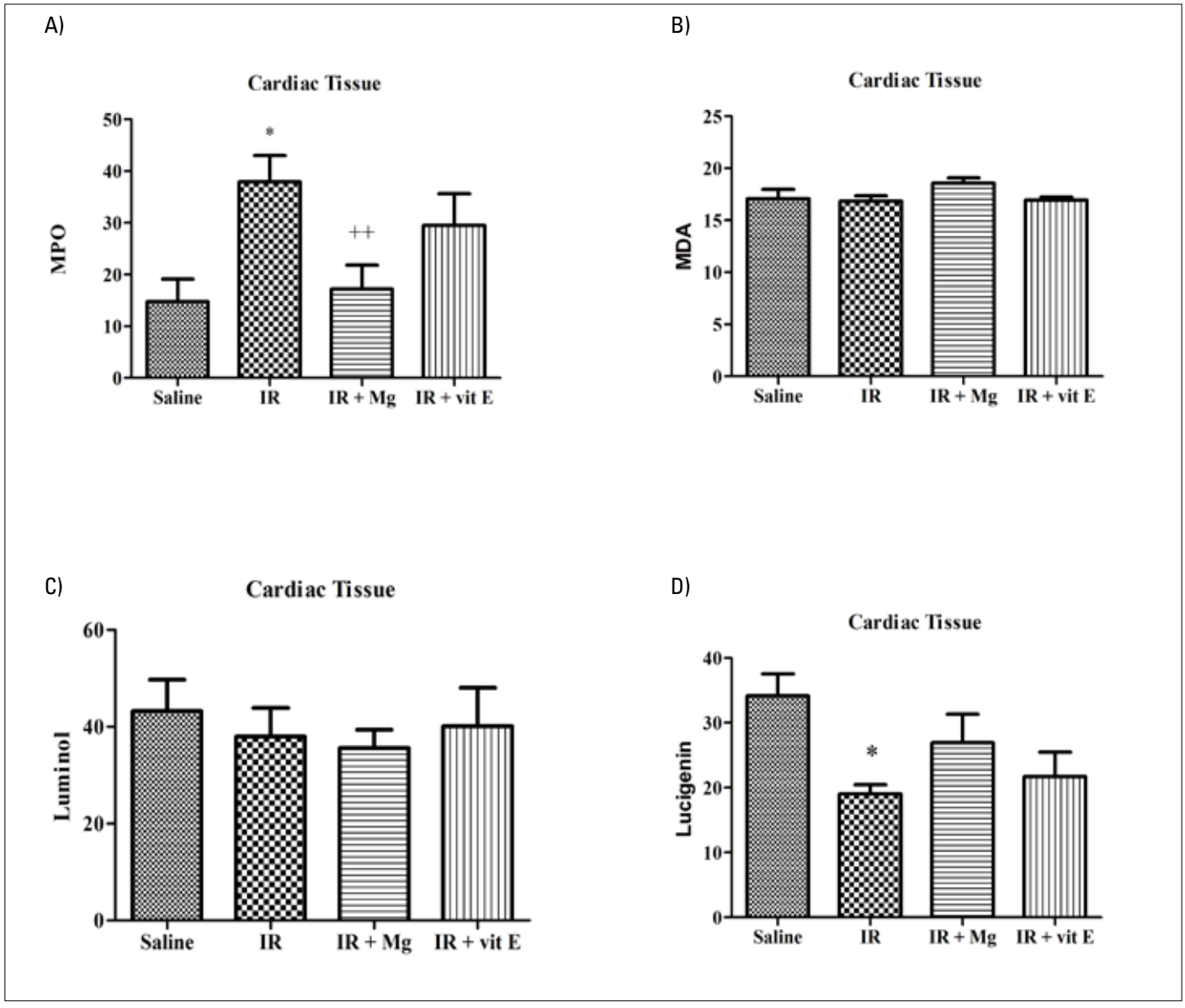

Figure 1. Effects of radiation and pretreatment with Mg and vit E on MPO, MDA, CL values in cardiac tissue samples

ANOVA with posthoc Bonferroni multiple comparison test

*p<0.01 compared with saline-treated group; ++p<0.05 compared with IR group

CL - chemiluminescence assay, IR- irradiation, MDA - malondialdehyde, Mg - magnesium sulphate, MPO - myeloperoxidase, vit - vitamin

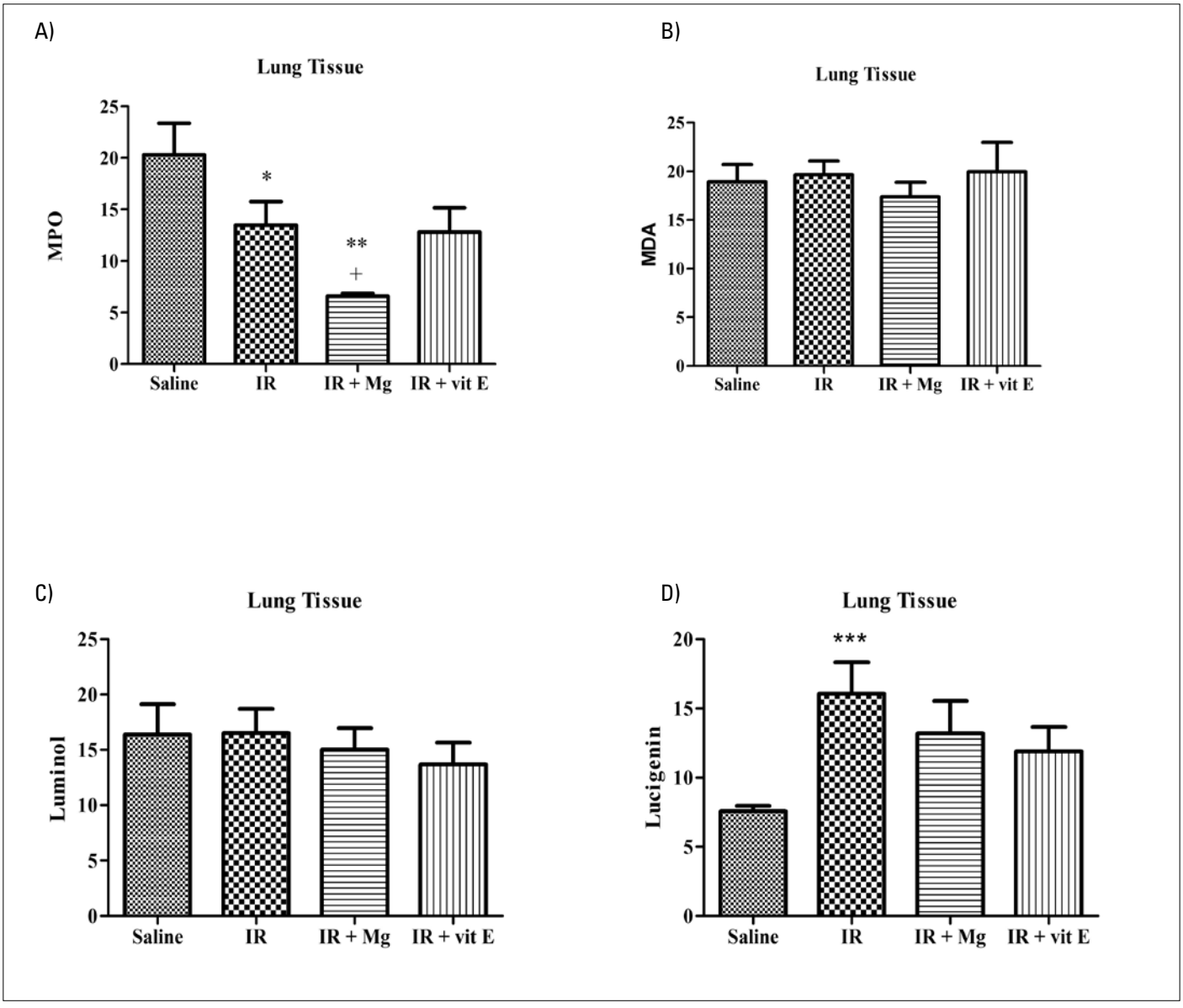

Figure 2. Effects of radiation and pretreatment with Mg and vit E on MPO, MDA, CL on values in lung tissue samples

ANOVA with posthoc Bonferroni multiple comparison test

*p<0.05 compared with saline-treated group; **p<0.001 and ***p<0.01 compared with saline-treated group; +p<0.05 compared with IR group

CL - chemiluminescence assay, IR- irradiation, MDA - malondialdehyde, Mg - magnesium sulphate, MPO - myeloperoxidase, vit - vitamin

cantly decreased the neutrophil accumulation on cardiac tissue and dramatically depressed pulmonary neutrophil infiltration.

The main issue that limits to order curative doses of radiotherapy in cancers of the chest cavity is the radiosensitivity of the normal cardiac and pulmonary tissues. Ionizing radiation induced lung damage, called as radiation pneumopathy, is a continuous process (4, 9, 10). Similarly, high cure rates of cancers and long follow-up times of young patients revealed that irradiation of the heart can cause chronic impairment of cardiac pump function and cardiac diseases (28, 29). Radiation ionizes water into reactive oxygen species (ROS) like $OH^{\cdot}$ and $H^{\cdot}$, which are responsible for most of the deleterious effects of ionizing radiation on tissues. ROS can attack DNA, membrane lipids and proteins. One of the characterized biological damages caused by ROS is their reaction with unsaturated lipids, known as lipid peroxidation (30). In their experimental study, Tokatlı et al. (31) have used amifostine, a potential ROS scavenger that is currently in use in radiotherapy. At the 100th day of irradiation, according to a myocardial degeneration grading, they observed a protective effect of amifostine on heart. Despite that amifostine (32) has a high level of evidence on its radioprotective effect in radiotherapy, its usage is not common because of the potential side effects such as hypotension, rush, nausea and vomiting, toxic epidermal necrolysis (33). Therefore, protectors with fewer side effects are required for the prevention against the long-term effects of radiation on the heart.

Despite of the relatively short-term follow-ups, it seems that encouraging the modern and more precise RT techniques may help to limit the side effects of radiation on normal cardiac tissue (34). Since some studies suggest that early responses to irradiation might be physiological and triggering inflammation may begin before the structural damage occurs (35), two different ROS scavengers, Mg and vitamin E were administered separately before the irradiation-induced lipid peroxidation was initiated. The results showed that at the 24-h of irradiation, pulmonary and cardiac tissues did not have irradiation-induced lipid peroxidation yet. However, during this early phase, one of the major sources of ROS and associated inflammatory mediators, neutrophils were recruited to the irradiated cardiac tissue, while Mg inhibited the infiltration of neutrophils. Observations suggest that ROS play a role in the recruitment of neutrophils into injured tissues, but activated neutrophils are also a potential source of ROS (36). Similarly, MPO activity of the pulmonary tissue was also reduced in Mg-pretreated irradiated rats, while enhanced generation of superoxide in the pulmonary tissue due to irradiation was not seen in Mg-pretreated groups. The results of our study indicate that magnesium sulfate has a reducing effect on neutrophil influx into the irradiated tissue. However, whether this reduction of neutrophil accumulation may have an anti-oxidant effect or may decrease the generation of lipid peroxidation is not observed in the present study, because at the 24-h of irradiation neither lung nor cardiac tissue demonstrated any irradiation-induced lipid peroxidation. Since IR-induced injury becomes evident at the 72nd h of irradiation (37), it is expected that this neutrophil-limiting effect of Mg could have a therapeutic value in the long term.

Our results showed that vit E did not have a preventive effect on the irradiation-induced cardiac or pulmonary injury. Similarly, Wiegman et al. (38) have used dietary vitamin E following irradiation and did not observe a preventive effect on radiation-induced lung fibrosis. They concluded that the development of radiation-induced lung damage was a complex process, consisting of both fibrosis and hyper-inflammation, minimizing the preventive effect of vit E alone on lung function (38). Thus, results revealed from vitamin E administration showed no benefit in terms of maintaining the endogenous anti-oxidant defense against radiation-induced injury neither in heart nor in lung in rats.

Study limitations

The major limitation of this study was evaluating the short term effects of radiation induced cardiac and pulmonary toxicity. Since ionizing radiation's effect is observed mostly in long- term period, we conclude that, for the short post-radiation duration of this study, pre-treatment with vitamin E was not capable of showing the reduction in radiation-induced lipid peroxidation. Tissue specification can be another issue to comment on. Kotzampassi et al. (39) reported their results on the preventive effect of vitamin E in liver, investigating the lipid peroxidation levels 2 and 6 h after RT in their study. Authors administered 25 mg/kg vitamin E 12 h and 1 h before irradiation. Despite the smaller doses and shorter post-radiation time, vitamin E led to a reduction in MDA in liver. Since heart is a relatively radioresistant organ and the signs require weeks to occur, we did not find any clinical signs or macroscopic changes at 24 h following RT administration.

Conclusion

According to our results, prophylactic use of magnesium sulfate has limited the infiltration of neutrophils to both the cardiac and pulmonary tissues at the 24 h of irradiation. However, how limiting neutrophils as the sources of ROS and inflammatory mediators would alter irradiation-induced lipid peroxidation and long-term injury needs to be studied. On the other hand, at the studied dose and time point, vitamin E did not seem to be of benefit in reducing irradiation-induced oxidative injury of lung and heart tissues. Further studies are required to identify the early and late effects of these anti-oxidant agents.

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References

1. Dörr W, Hendry JH. Consequential late effects in normal tissues. Radiother Oncol 2001; 61: 223-31. [CrossRef]
2. McGale P, Darby SC, Hall P, Adolfsson J, Bengtsson NO, Bennet AM, et al. Incidence of heart disease in 35.000 women treated with radiotherapy for breast cancer in Denmark and Sweden. Radiother Oncol 2011; 100: 167-75. [CrossRef]
3. Paszat LF, Mackillop WJ, Groome PA, Boyd C, Schulze K, Holowaty E. Mortality from myocardial infarction after adjuvant radiotherapy for breast cancer in the surveillance, epidemiology, and end-results cancer registries. J Clin Oncol 1998; 16: 2625-31.
4. Ng AK. Review of the cardiac long-term effects of therapy for Hodgkin lymphoma. Br J Haematol 2011; 154: 23-31. [CrossRef]
5. Citrin D, Cotrim AP, Hyodo F, Baum BJ, Krishna MC, Mitchell JB. Radioprotectors and mitigators of radiation-induced normal tissue injury. Oncologist 2010; 15: 360-71. [CrossRef]
6. Darby SC, Cutter DJ, Boerma M, Constine LS, Fajardo LF, Kodama K, et al. Radiation-related heart disease: current knowledge and future prospects. Int J Radiat Oncol Biol Phys 2010; 76: 656-65. [CrossRef]
7. Schultz-Hector S, Trott KR. Radiation-induced cardiovascular diseases: is the epidemiologic evidence compatible with the

radiobiologic data? Int J Radiat Oncol Biol Phys 2007; 67: 10-8. [CrossRef]
8. Andratschke N, Maurer J, Molls M, Trott KR. Late radiation-induced heart disease after radiotherapy. Clinical importance, radiobiological mechanisms and strategies of prevention. Radiother Oncol 2011; 100: 160-6. [CrossRef]
9. Trott KR, Herrmann T, Kasper M. Target cells in radiation pneumopathy. Int J Radiat Oncol Biol Phys 2004; 58: 463-9. [CrossRef]
10. Eberhardt W, Pöttgen C, Stuschke M. Chemoradiation paradigm for the treatment of lung cancer. Nat Clin Pract Oncol 2006; 3: 188-99. [CrossRef]
11. Graves PR, Siddiqui F, Anscher MS, Movsas B. Radiation pulmonary toxicity: from mechanisms to management. Semin Radiat Oncol 2010; 20: 201-7. [CrossRef]
12. McLean RM. Magnesium and its therapeutic uses: a review. Am J Med 1994; 96: 63-76. [CrossRef]
13. Dickens BF, Weglicki WB, Li YS, Mak IT. Magnesium deficiency in vitro enhances free radical-induced intracellular oxidation and cytotoxicity in endothelial cells. FEBS Lett 1992; 311: 187-91. [CrossRef]
14. Regan RF, Jasper E, Guo Y, Panter SS. The effect of magnesium on oxidative neuronal injury in vitro. J Neurochem 1998; 70: 77-85. [CrossRef]
15. Lang-Lazdunski L, Heurteaux C, Dupont H, Widmann C, Lazdunski M. Prevention of ischemic spinal cord injury: comparative effects of magnesium sulfate and riluzole. J Vasc Surg 2000; 32: 179-89. [CrossRef]
16. Fang YZ, Yang S, Wu G. Free radicals, antioxidants, and nutrition. Nutrition 2002; 18: 872-9. [CrossRef]
17. Beşe NS, Munzuroğlu F, Uslu B, Arbak S, Yeşiladalı G, Süt N, et al. Vitamin E protects against the development of radiation-induced pulmonary fibrosis in rats. Clin Oncol (R Coll Radiol) 2007; 19: 260-4. [CrossRef]
18. Empery LR, Papp JD, Jewell LD, Fedorak RN. Mucosal protective effects of vitamin E and misoprostol during acute radiation-induced enteritis in rats. Dig Dis Sci 1992; 37: 205-14. [CrossRef]
19. Krüse JJ, Zurcher C, Strootman EG, Bart CI, Schlagwein N, Leer JW, et al. Structural changes in the auricles of the rat heart after local ionizing irradiation. Radiother Oncol 2001; 58: 303 -11. [CrossRef]
20. Epperly MW, Travis EL, Sikora C, Greenberger JS. Manganese [correction of Magnesium] superoxide dismutase (MnSOD) plasmid/liposome pulmonary radioprotective gene therapy: modulation of irradiation-induced mRNA for IL-I, TNF-alpha, and TGF-beta correlates with delay of organizing alveolitis/fibrosis. Biol Blood Marrow Transplant 1999; 5: 204-14 [CrossRef]
21. Bradley PP, Priebat DA, Christersen RD, Rothstein G. Measurement of cutaneous inflammation: estimation of neutrophil content with an enzyme marker. J Invest Dermatol 1982; 78: 206-9. [CrossRef]
22. Hillegass LM, Griswold DE, Brickson B, Albrightson-Winslow C. Assessment of myeloperoxidase activity in whole rat kidney. J Pharmacol Methods 1990; 24: 285-95. [CrossRef]
23. Yagi K. Assay for blood plasma or serum. Methods in Enzymol 1984; 105: 328-31. [CrossRef]
24. Ohara Y, Peterson TE, Harrisorn DG. Hypercholesterolemia increases endothelial superoxide anion production. J Clin Invest 1993; 91: 2546-51. [CrossRef]
25. Davies GR, Simmonds NJ, Stevens TR, Sheaff MT, Banatvala N, Laurenson IF, et al. Helicobacter pylori stimulates antral mucosal reactive oxygen metabolite production in vivo. Gut 1994; 35: 179-85. [CrossRef]
26. Haklar G, Sayın-Özveri E, Yüksel M, Aktan AO, Yalçın AS. Different kinds of reactive oxygen and nitrogen species were detected in colon and breast tumors. Cancer Lett 2001; 165: 219-24. [CrossRef]
27. Haklar U, Yüksel M, Velioğlu A, Türkmen M, Haklar G, Yalçın AS. Oxygen radicals and nitric oxide levels in chondral or meniscal lesions or both. Clin Orthop Relat Res 2002; 403: 135-42. [CrossRef]
28. Noel G, Mazeron JJ. Favorable and unfavorable effects on long-term survival of radiotherapy for early breast cancer: an overview of randomized trials. Cancer Radiother 2001; 5: 92-4. [CrossRef]
29. Lee CK, Aeppli D, Nierengarten ME. The need for long-term surveillance for patients treated with curative radiotherapy for Hodgkin's disease: University of Minnesota experience. Int J Radiat Oncol Biol Phys 2000; 48: 169-79. [CrossRef]
30. Hall EJ, Giaccia AM. Physics and chemistry of radiation absorption. In: Hall EJ, Giaccia AM, editors. Radiobiology for Radiologist. 6th edition. Philadelphia; Lipincott Williams&Wilkins: 2006. p. 11-4.
31. Tokatlı F, Uzal C, Doğanay L, Koçak Z, Kaya M, Türe M, et al. The potential cardioprotective effects of amifostine in irradiated rats. Int J Radiat Oncol Biol Phys 2004; 58: 1228-34. [CrossRef]
32. Wasserman TH, Brizel DM, Henke M, Monnier A, Eschwege F, Sauer R, et al. Influence of intravenous amifostine on xerostomia, tumor control, and survival after radiotherapy for head-and- neck cancer: 2-year follow-up of a prospective, randomized, phase III trial. Int J Radiat Oncol Biol Phys 2005; 63: 985-90. [CrossRef]
33. Demiral AN, Yerebakan O, Şimşir V, Alpsoy E. Amifostine-induced toxic epidermal necrolysis during radiotherapy: a case report. Jpn J Clin Oncol 2002; 32: 477-9. [CrossRef]
34. Demirci S, Nam J, Hubbs JL, Nguyen T, Marks LB. Radiation-induced cardiac toxicity after therapy for breast cancer: interaction between treatment era and follow-up duration. Int J Radiat Oncol Biol Phys 2009; 73: 980-7. [CrossRef]
35. MacNaughton WK. Review article: new insights into the pathogenesis of radiation-induced intestinal dysfunction. Aliment Pharmacol Ther 2000; 14: 523-8. [CrossRef]
36. Kettle AJ, Winterbourn CC. Myeloperoxidase: a key regulator of neutrophil oxidant production. Redox Report 1997; 3: 3-15.
37. Şener G, Kabasakal L, Atasoy BM, Erzik C, Velioğlu-Öğünç A, Çetinel S, et al. Propylthiouracil-induced hypothyroidism protects ionizing radiation-induced multiple organ damage in rats. J Endocrinol 2006; 189: 257-69. [CrossRef]
38. Wiegman EM, van Gameren MM, Kampinga HH, Szabo BG, Coppes RP. Post-irradiation dietary vitamin E does not affect the development of radiation-induced damage in rats. Radiother Oncol 2004; 72: 67-70. [CrossRef]
39. Kotzampassi K, Paramythiotis D, Tsiakitzis K, Kourounakis AP, Tzitzikas J, Metaxas G, et al. The impact of α-tocopherol on radiation-induced liver injury. Nutrition Research 2003; 23: 103-9. [CrossRef]