

Received: 2014.01.11
Accepted: 2014.01.26
Published: 2014.02.21

The relation of radiation-induced pulmonary fibrosis with stress and the efficiency of antioxidant treatment: An experimental study

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

ABCDEF 1 **Vildan Kaya**
ABD 2 **Rasih Yazkan**
CEF 3 **Mustafa Yıldırım**
BD 4 **Duygu Kumbul Doğuç**
BD 5 **Dinç Süren**
BD 6 **Kemal Kürşat Bozkurt**
BD 4 **Özlem Yüksel**
CD 7 **Özlem Demirpence**
DF 8 **Cenk Ahmet Şen**
BFG 1 **Ayşen Yeşim Yalçın**

1 Department of Radiation Oncology, Süleyman Demirel University School of Medicine, Isparta, Turkey
2 Department of Chest Surgery, Süleyman Demirel University School of Medicine, Isparta, Turkey
3 Department of Medical Oncology, Ministry of Health Batman Regional Government Hospital, Batman, Turkey
4 Department of Biochemistry, Süleyman Demirel University School of Medicine, Isparta, Turkey
5 Department of Pathology, Antalya Education and Research Hospital, Antalya, Turkey
6 Department of Pathology, Süleyman Demirel University School of Medicine, Isparta, Turkey
7 Department of Biochemistry, Ministry of Health Batman Regional Government Hospital, Batman, Turkey
8 Department of Radiation Oncology, Izmir University School of Medicine, Izmir, Turkey

Corresponding Author: Vildan Kaya, e-mail: vildansimsir@yahoo.com
Source of support: Departmental sources

Background: Radiation-Induced Lung Injury has 2 components: radiation pneumonitis and radiation fibrosis. The pulmonary fibrosis has no known efficient treatment. The purpose of this study was to study the relationship between the oxidant/antioxidant status and pulmonary fibrosis in rats having radiation induced pulmonary fibrosis and to study the antioxidant effects of pentoxifylline, vitamin E, and vitamin C in the treatment of pulmonary fibrosis.





Material/Methods: The study rats were divided into 5 groups: Thoracic RT + vitamin E+ Pentoxifylline for group 1, Thoracic RT + vitamin C + Pentoxifylline for group 2, Thoracic RT + vitamin C + vitamin E + Pentoxifylline for group 3, and Thoracic RT + Pentoxifylline for group 4, and group 5 was the control group.

Results: When groups are evaluated in pairs, significant differences between group 1 and 2, group 1 and 4, and group 1 and 5 were determined (p: 0.002, p: 0.002, p<0.001, respectively). No significant difference was determined between group 1 and 3 (p: 0.161). No significant difference was determined between group 2 and group 3, 4, and 5 (p: 0.105, p: 0.645, p: 0.234, respectively). There was no significant difference between group 4 and 5 (p: 0.645).

Conclusions: The combination of vitamin E and pentoxifylline is efficient in preventing radiation-induced lung fibrosis. The additional benefit of vitamin C, which is added to this combination to increase the antioxidant activity, cannot be shown. It would be useful to investigate the combination of vitamin E, pentoxifylline, and other non-enzymatic antioxidants.

MeSH Keywords: **Pulmonary Fibrosis • Oxidative Stress • Radiation, X • Vitamin E • Pentoxifylline**

Full-text PDF: <http://www.medscimonit.com/download/index/idArt/890334>

 2479  1  5  30

1 Background

Thoracic radiotherapy is an important treatment modality that can be used concomitant with chemotherapy or alone in the neoadjuvant, adjuvant, and palliative treatment setting of lung, breast, esophagus, and lymphatic system cancer treatments. The lung is one of the most radiosensitive organs. Therefore, pulmonary toxicity is an important dose-limiting factor for thoracic radiotherapy [1].

Radiation-Induced Lung Injury (RILI) has 2 components: radiation pneumonitis (RP) and radiation fibrosis (RF). While RP appears in the first stages of the damage, fibrosis is formed in a later period [2]. The alveolar epithelium is composed of Type 1 and Type 2 pneumocytes. The epithelial Type 1 forms 90% of the alveolar epithelium in the normal lung tissue. Toxic damage leads a decrease in number of Type 1 pneumocytes, but Type 2 pneumocytes increase. Type 2 pneumocytes contribute to inflammation through several proteases and growth factors [3].

Radiation causes direct vascular damage. Vascular damage, activation of the coagulation cascade, and oxidative stress with conjunction triggers the development of radiation-induced pulmonary fibrosis (RIPF) [4,5]. The subclinical damage of Type 1 pneumocytes in early stages causes acute interstitial inflammation. Progression of this damage clinically stands out as radiation pneumonitis. In the process of recovery from radiation pneumonia, fibrosis occurs due to the uncontrolled recovery pattern. The fibrosis may also develop in the absence of specific clinical pneumonitis [6].

Vitamin E is an important vitamin active in defense against oxidative stress and it plays an important role in preventing lipid peroxidation in the cellular membrane [7]. Vitamin E has been found to be effective in preventing radiation-induced gastrointestinal injury [8]. A xanthine derivative of pentoxifylline is the phosphodiesterase inhibitor. In addition to the vasodilator effects of pentoxifylline, it also has antioxidant and anti-inflammatory effects [9]. It was shown that the combination of vitamin E and pentoxifylline prevents radiation-induced skin injury [10]. Vitamin C is a well-known, potent scavenger of reactive oxygen species in plasma and it has been suggested that vitamin C prevents hepatic fibrosis by inhibiting NF- κ B [11].

Pulmonary fibrosis causes a loss of pulmonary functions, leading to substantial morbidity in patients. To date, no efficient treatment has been found for pulmonary fibrosis [12]. The current alternatives in practical use are mostly anti-inflammatory and immunosuppressive agents [13]. These all have low impact despite the relatively serious adverse effects. The purpose of this study was to investigate the relationship between total oxidant status (TOS), which is an oxidative stress indicator, total antioxidant capacity (TAC), oxidative stress index (OSI), and pulmonary

fibrosis in rats with radiation-induced pulmonary fibrosis and to evaluate the effectiveness of pentoxifylline, vitamin E, and vitamin C as antioxidants in the treatment of pulmonary fibrosis.

5 Material and Methods

Animals

Albino Wistar rats with an average age of 6–8 months were obtained from the Laboratory of Experimental Animal Production and Experimental Research of the Suleyman Demirel University School of Medicine. The mean weight of the animals was 0.330 kg. The care and the nutrition of the animals during the study were provided by the Laboratory of Experimental Animal Production and Experimental Research of the University of Suleyman Demirel. Animals were treated according to the European Convention on Animal Care. The study was approved by the Local Ethics Board for Animal Experiments of the University of Suleyman Demirel University School of Medicine.

Working groups

The animals in the study were randomized into 5 groups. Each group had 8 animals. Groups 1, 2, 3, and 4 were designated as working groups and group 5 was the control group. group 1 was given Thoracic RT + vitamin E+ Pentoxifylline, group 2 was given Thoracic RT + vitamin C + Pentoxifylline, group 3 was given Thoracic RT + vitamin C + vitamin E + Pentoxifylline, and group 4 was given Thoracic RT + Pentoxifylline. The control group (Group 5) received only Thoracic RT.

The scheme of radiotherapy and antioxidant delivery

A total Thoracic RT of 14 Gy was applied as an only AP area, on 1 fraction under general anesthesia (xylazine 5 mg/kg and ketamine 50 mg/kg, intramuscular) with the linear accelerator device (VARIAN DHX) by using 6 MV photon energy. One day before the RT implementation, the CT was taken for planning the RT and the treatment plan was made. The implementation of vitamin E, Pentoxifylline, and vitamin C started the first day after the RT. Pentoxifylline (Trental, Sanofi Aventis, Canada) was applied 3.4 mg/day orally, vitamin E (Evicap, Koçak Farma, Turkey) was applied 20 mg/kg/day orally, and vitamin C (Redoxon, Bayer, Germany) was applied 75 mg/kg/day orally. The antioxidant substances are given by gavage 7 days a week, during 12 weeks, each at the same hour, together with 1 researcher and 1 technician from the laboratory of experimental animals.

Obtaining samples and histopathological examination

Animals were sacrificed under general anesthesia 12 weeks after the RT. Blood was taken from the abdominal aorta. Right

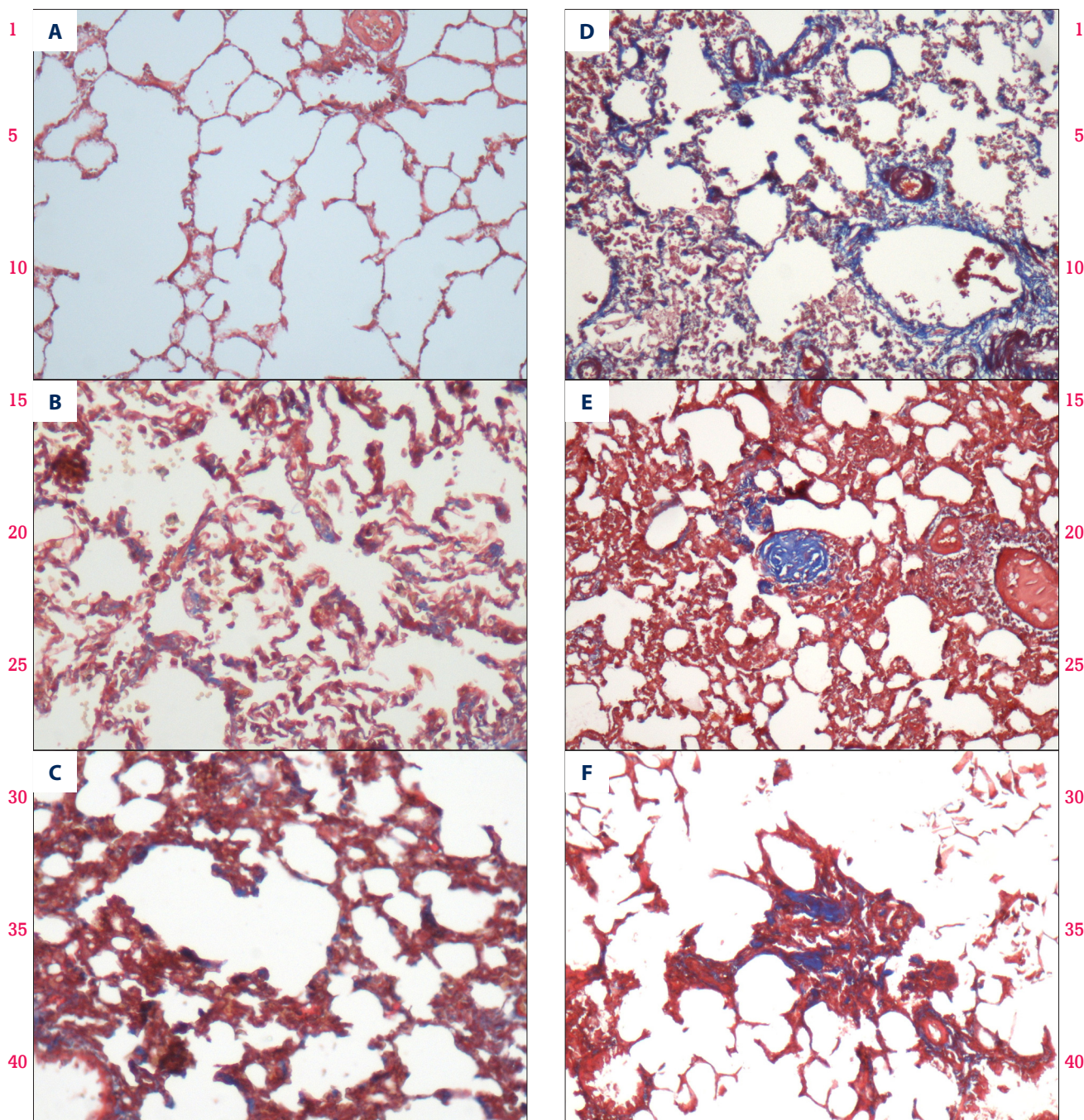


Figure 1. (A) Grade 0, normal lung (Masson's trichrome $\times 100$). (B) Grade 1, isolated alveolar septa with gentle fibrotic changes (Masson trichrome $\times 200$). (C) Grade 2, fibrotic changes of alveolar septa with knot-like formation (Masson trichrome $\times 200$). (D) Grade 3, contiguous fibrotic walls of alveolar septa. (Masson trichrome $\times 100$). (E) Grade 4, single fibrotic mass (Masson trichrome $\times 100$). (F) Grade 5, confluent fibrotic masses (Masson trichrome $\times 100$).

and left lungs were dissected and fixed in 10% neutral formalin for histopathological examination and were embedded to paraffin. Lung histological specimens (5 μ m) were stained with hematoxylin/eosin (H&E) for morphology and Masson's trichrome to show collagen deposits. The histopathological examination was done by 2 pathologists. The fibrosis scoring was

accomplished according to the fibrosis scoring recommended by Hübner et al. (Figure 1A–F) [14]. The total fibrosis score was obtained from the sum of the left and right lung fibrosis score. Blood samples were centrifuged for 5 min at 5000 rpm and serum fractions were obtained. The serum samples were portioned and were kept at -80°C until the time of the study.

Table 1. Fibrosis score, TAC, TOS, OSI according to the groups.

Group	Fibrosis Score				TAS	TOS	OSI
	Right lung		Left lung				
	Median ±SD	Range	Median ±SD	Range			
Group 1 (RT+Vit E+Pentoxifylline)	1.5±0.53	1–2	1.5±0.53	1–2	1.4±0.3	16±3.8	11.5±2.7
Group 2 (RT+ Vit C+ Pentoxifylline)	3±0.92	2–5	3±1.06	2–5	1.8±0.6	13.1±3.9	8.2±4.1
Group 3 (RT+Vit C+Vit E+ Pentoxifylline)	2±0.83	1–3	2±0.83	1–3	1.8±0.7	11.2±1.3	6.8±2.2
Group 4 (RT+ Pentoxifylline)	3±1.04	2–5	3±0.53	2–4	1.2±0.2	25.1±11.5	20±11.1
Group 5 (RT)	3±0.75	3–5	3±0.51	3–4	1±0.2	23.2±9.1	22.4±10.2

TAC – total antioxidant capacity; TOS – total oxidant status; OSI – oxidative stress index.

Measurement of the total antioxidant capacity

TAC of serum was determined using a novel automated measurement method developed by Erel [15]. In this method, hydroxyl radical, which is the most potent biological radical, is produced. In the assay, ferrous ion solution, which is present in Reagent 1, is mixed by hydrogen peroxide, which is present in Reagent 2. The sequentially produced radicals, such as brown-colored dianisidiny radical cation, produced by the hydroxyl radical, are also potent radicals. Using this method, antioxidative effect of the sample is measured against the potent free radical reactions, which is initiated by the produced hydroxyl radical. The assay has excellent precision values lower than 3%. The results are expressed as mmol Trolox Equiv./L.

Measurement of total oxidant status

Total oxidant status was measured by Erel's methods [16]. Oxidants present in the sample oxidize the ferrous ion-o-dianisidine complex to ferric ion. The oxidation reaction is enhanced by glycerol molecules, which are abundantly present in the reaction medium. The ferric ion makes a colored complex with xylenol orange in an acidic medium. The color intensity, which can be measured spectrophotometrically, is related to the total amount of oxidant molecules present in the sample. The assay is calibrated with hydrogen peroxide and the results are expressed in terms of micromolar hydrogen peroxide equivalent per liter ($\mu\text{mol H}_2\text{O}_2$ Equiv./L).

Oxidative stress index

Percentage ratio of TOS level to TAC level was accepted as OSI. The OSI value was calculated according to the formula: $\text{OSI (Arbitrary Unit)} = \text{TOS (}\mu\text{mol H}_2\text{O}_2 \text{ Equiv./L)}/\text{TAC (mmol Trolox Equiv./L)}$ [17].

Statistical analysis

Statistical analyses were done using SPSS for Windows 15.0 software. Visual (histograms and probability plots) and analytical methods (Kolmogorov-Smirnov/Shapiro-Wilk tests) were used to determine if the variables were normally distributed. The fibrosis score between the groups TAC, TOS, and OSI (since the fibrosis scoring is an ordinal variable and since TAC, TOS and OSI are not normally distributed) were investigated by using the Kruskal-Wallis test. Comparisons in pairs were evaluated by using the Mann-Whitney U test and the Bonferroni correction method. The Hübner scores obtained from the right and left lungs of the same experimental subject were compared using the Wilcoxon test. The total Type 1 error level of 5% was utilized for statistical significance.

Results

When all the groups were evaluated together, the fibrosis score of the right lung was determined to be 3 ± 1.09 and the fibrosis score of the left lung was determined to be 3 ± 0.98 (Table 1). No difference was determined between the right and left lung in terms of the fibrosis score ($p=0.477$). The total fibrosis score was determined to be 5.27 ± 1.97 (range, 2–10). A significant difference between the groups was found in terms of the total fibrosis score ($p<0.001$) (Figure 2).

When groups were evaluated in pairs, statistically significant differences between group 1 and 2, group 1 and 4, and group 1 and 5 were determined ($p=0.001$, $p=0.001$, $p<0.001$ the significant p value is accepted as 0.01 with the Bonferroni correction). No significant difference is determined between group 1–3 ($p=0.103$). No significant difference is determined between group 2 and group 3, 4, 5 ($p=0.089$, $p=0.440$, and $p=0.196$, respectively). While there was no difference between group

PROOF © INTERNATIONAL SCIENTIFIC INFORMATION

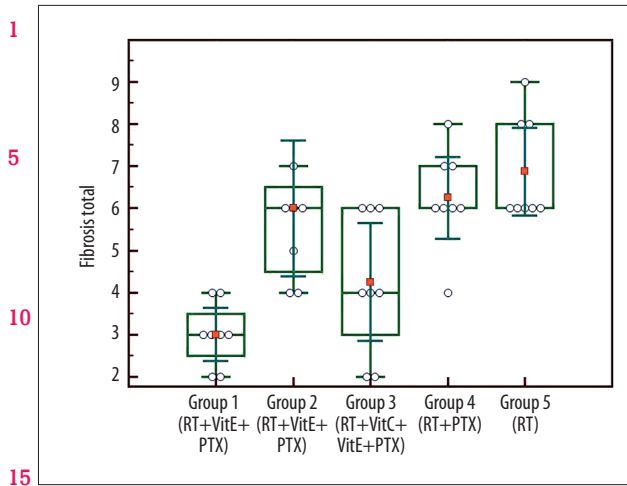


Figure 2. According to groups total fibrosis score (RT – thoracic radiotherapy, PTX – pentoxifylline).

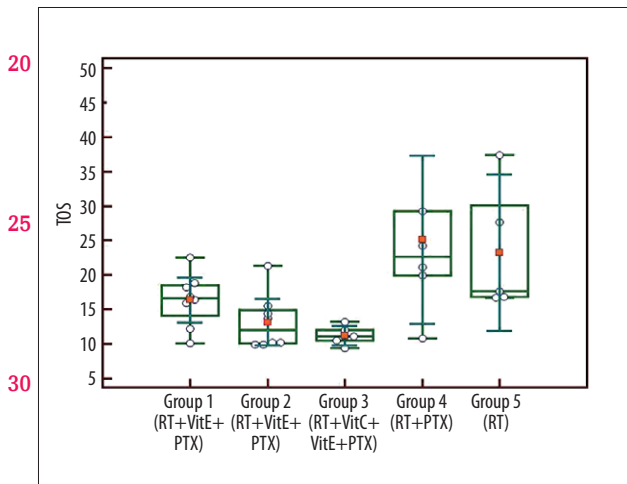


Figure 3. According to groups total antioxidant capacity.

3 and group 4, a significant difference was found with group 5 ($p=0.017$, $p=0.006$). There was no significant difference between group 4 and group 5 ($p=0.485$).

When all the groups were evaluated together, TAC was determined as 1.5 ± 0.54 . The TAC values between groups were statistically significantly different ($p=0.015$) (Figure 3). No significant difference was determined between group 1 and group 2, 3, 4, and 5 ($p=0.279$, $p=0.282$, $p=0.345$, and $p=0.19$, respectively).

No significant difference was determined between group 2 and group 3 and group 4 ($p=0.1$, $p=0.081$). A significant difference was determined between group 2 and group 5 ($p=0.006$). No significant difference was determined between group 3 and group 4 and group 5 ($p=0.093$, $p=0.017$). No significant difference was determined between group 4 and group 5 ($p=0.052$).

The TOS value was determined as 17.2 ± 8.1 for all groups. A significant difference was determined between the groups in

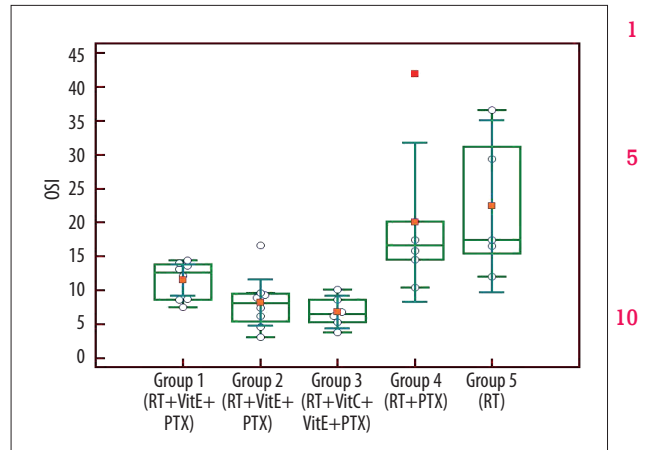


Figure 4. According to groups total oxidant status.

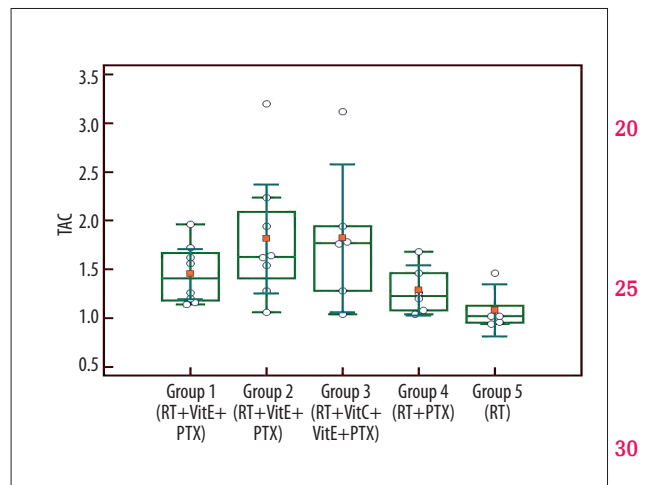


Figure 5. According to groups oxidative stress index.

terms of the TOS value ($p=0.004$) (Figure 4). No significant difference was determined between group 1 and group 2, 3, 4, and 5 ($p=0.083$, $p=0.82$, $p=0.059$, and $p=0.171$, respectively). No significant difference was determined between group 2 and group 3, 4, 5 ($p=0.662$, $p=0.02$, $p=0.011$). While there was no difference between group 3 and group 4, a significant difference was found with group 5 ($p=0.026$, $p=0.004$).

When OSI was evaluated in the entire group, it was determined as 13 ± 8.6 . A significant difference was determined between the groups in terms of the TOS value ($p=0.001$) (Figure 5). No significant difference was determined between group 1 and group 2, 3, 4, and 5 ($p=0.13$, $p=0.013$, $p=0.013$, and $p=0.03$, respectively). There was no difference between group 2 and group 3, but a significant difference was found with group 4 and group 5 ($p=0.573$, $p=0.005$, and $p=0.006$, respectively). While a significant difference was determined between group 3 and group 4, no significant difference was found with group 5 ($p=0.002$, $p=0.084$). No significant difference was determined between group 4 and group 5 ($p=0.662$).

1 Discussion

We determined that the combination of vitamin E and pentoxifylline decreases fibrosis when compared to the combination of vitamin C and pentoxifylline, pentoxifylline only, or to not using any antioxidant. We also found that adding vitamin C to the combination of vitamin E and pentoxifylline does not improve the result. Combination of vitamin C and pentoxifylline was shown to be ineffective in reducing fibrosis.

PTX in the concentration used was ineffective in reducing fibrosis when compared to not using any agent. Immunomodulatory and anti-inflammatory effects of PTX at doses higher than those used in our study have been reported [18]. Using that dose of PTX can reduce the fibrosis.

These results lead us to conclude that the combination of vitamin E and pentoxifylline does not use the antioxidant system as its mechanism of action. Although we determined the highest TAC value in the vitamin C and pentoxifylline combination, this combination was ineffective in preventing fibrosis. However, oxidative stress and endothelial damage may lead to DNA damage in cells [19,20].

In their studies investigating the effectiveness of the combination of vitamin E and pentoxifylline in rats, Bese et al. reported that vitamin E prevents radiation-induced lung fibrosis (RILF) and that adding pentoxifylline has no additional impact [21]. In that study, oral pentoxifylline was used in a dose similar to that used in our study. Vitamin E is a fat-soluble vitamin and is absorbed with lipids when it is taken orally. It was shown that pentoxifylline acutely affects intestinal glucose absorption and lipid metabolism [22]. Pentoxifylline may affect the bioavailability of vitamin E and may contribute to its effect in preventing fibrosis.

A higher dose of vitamin E does not cause a greater fibrosis-preventive effect. A study in which vitamin E was used in higher doses (62.7 mg/kg) than in our study failed to demonstrate the effect of prevention of RILF in rats [23].

Transforming growth factor β (TGF- β) plays a role in the regulations of cytokines, which are synthesized in many cell types and also have proliferation, differentiation, and many immunomodulatory effects. TGF- β also regulates the production of extracellular matrix components. Increase in TGF- β was shown to play a role in radiation-induced pulmonary damage [24].

References:

1. Kocak Z, Evans ES, Zhou et al: Challenges in defining radiation pneumonitis in patients with lung cancer. *Int J Radiat Oncol Biol Phys*, 2005; 62: 635–38

Hamama et al. reported that the combination of vitamin E and pentoxifylline decreases the severity of radiotherapy-induced enteropathy syndromes and that this effect was possibly due to the inhibition of TGF- β gene expression [25]. The fact that the addition of vitamin C to the combination of vitamin E and pentoxifylline had no additional effect in our study shows that the anti-fibrotic activity is developed through a pathway other than the antioxidant pathway. This is also supported by the fact that different TAC and TOS values are not related with the fibrosis score.

In addition, antioxidant treatments decrease RILF significantly. Pan et al. showed that recombinant protein superoxide dismutase-TAT (SOD-TAT) increases pulmonary antioxidant activity and prevents RILF [26].

The accumulation of reactive oxygen products is one of the known causes of RILF [27]. Vitamin C has strong antioxidant effects. Vitamin C chromium-induced pulmonary fibrosis prevents pulmonary fibrosis in rats and its combination with vitamin E is more effective [28]. On the other hand, it was determined that vitamin C is not effective in preventing fibrosis created with paraquat [29]. It was shown that nonalcoholic steatohepatitis-related fibrosis decreases in patients when a vitamin C and vitamin E combination is applied compared to placebo. In the present study, we found that the addition of vitamin C to the treatment in RILF does not have any advantage [30], perhaps because the fibrosis process is created by different mechanisms.

Conclusions

As a result, the combination of vitamin E and pentoxifylline is efficient in preventing RILF, which is one of the important restrictive factors in thoracic radiotherapy. The additional benefit of vitamin C, which was added to this combination to increase the antioxidant activity, cannot be shown. It would be useful to investigate the combination of vitamin E, pentoxifylline, and other non-enzymatic antioxidants.

Acknowledgements

We have no financial interest or conflict of interest in association with this work. This manuscript has not been published previously and is not being considered for publication by another journal. All authors have read and approved the final manuscript.

2. Brickey WJ, Neuringer IP, Walton W et al: MyD88 provides a protective role in long-term radiation-induced lung injury. *Int J Radiat Biol*, 2012; 88: 335–47

- 1 3. Kasper M, Haroske G: Alterations in the alveolar epithelium after injury leading to pulmonary fibrosis. *Histol Histopathol*, 1996; 11: 463–83
4. Chen Y, Williams J, Ding I et al: Radiation pneumonitis and early circulatory cytokine markers. *Semin Radiat Oncol*, 2002; 12: 26–33
- 5 5. Abushamaa AM, Sporn TA, Folz RJ: Oxidative stress and inflammation contribute to lung toxicity after a common breast cancer chemotherapy regimen. *Am J Physiol Lung Cell Mol Physiol*, 2002; 283: L336–45
6. Graves PR, Siddiqui F, Anscher MS et al: Radiation pulmonary toxicity: from mechanisms to management. *Semin Radiat Oncol*, 2010; 20: 201–7
7. Marquardt D, Williams JA, Kučerka N et al: Tocopherol activity correlates with its location in a membrane: a new perspective on the antioxidant vitamin E. *J Am Chem Soc*, 2013; 135: 7523–33
- 10 8. Empley LR, Papp JD, Jewell LD et al: Mucosal protective effects of vitamin E and misoprostol during acute radiation-induced enteritis in rats. *Dig Dis Sci*, 1992; 37: 205–14
9. Lloris Carsi JM, Cejalvo Lapeña D, Toledo AH et al: Pentoxifylline protects the small intestine after severe ischemia and reperfusion. *Exp Clin Transplant*, 2013; 11: 250–58
- 15 10. Haddad P, Kalaghchi B, Amouzegar-Hashemi F: Pentoxifylline and vitamin E combination for superficial radiation-induced fibrosis: a phase II clinical trial. *Radiother Oncol*, 2005; 77: 324–26
11. Abhilash PA, Harikrishnan R, Indira M: Ascorbic acid suppresses endotoxemia and NF- κ B signaling cascade in alcoholic liver fibrosis in guinea pigs: A mechanistic approach. *Toxicol Appl Pharmacol*, 2014; 274: 215–24
- 20 12. Cooper JA: Pulmonary fibrosis: pathways are slowly coming into light. *Am J Respir Cell Mol Biol*, 2000; 22: 520–23
13. Fell CD, Martinez FJ, Liu LX et al: Clinical predictors of a diagnosis of idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med*, 2010; 181: 832–37
14. Hübner RH, Gitter W, El Mokhtari NE et al: Standardized quantification of pulmonary fibrosis in histological samples. *Biotechniques*, 2008; 44: 507–11
15. Erel O: A novel automated method to measure total antioxidant response against potent free radical reactions. *Clin Biochem*, 2004; 37: 112–19
- 25 16. Erel O: A new automated colorimetric method for measuring total oxidant status. *Clin Biochem*, 2005; 38: 1103–11
17. Karaagac L, Koruk ST, Koruk I et al: Decreasing oxidative stress in response to treatment in patients with brucellosis: could it be used to monitor treatment? *Int J Infect Dis*, 2011; 15: 346–49
18. Lazarczyk M, Grzela T, Niderla J et al: Differential influence of pentoxifylline on murine colon adenocarcinoma- and melanoma-derived metastatic tumor development in lungs. *Oncol Rep*, 2004; 11: 1121–25
19. Płachetka A, Adamek B, Strzelczyk JK et al: 8-hydroxy-2'-deoxyguanosine in colorectal adenocarcinoma—is it a result of oxidative stress? *Med Sci Monit*, 2013; 19: 690–95
- 5 20. Bartnicki P, Fijałkowski P, Majczyk M et al: Effect of methoxy polyethylene glycol-epoetin beta on oxidative stress in predialysis patients with chronic kidney disease. *Med Sci Monit*, 2013; 19: 954–59
21. Bese NS, Munzuroglu F, Uslu B et al: Vitamin E protects against the development of radiation-induced pulmonary fibrosis in rats. *Clin Oncol (R Coll Radiol)*, 2007; 19: 260–64
22. Massart J, Robin MA, Noury F et al: Pentoxifylline aggravates fatty liver in obese and diabetic ob/ob mice by increasing intestinal glucose absorption and activating hepatic lipogenesis. *Br J Pharmacol*, 2012; 165: 1361–74
23. Wiegman EM, von Gameren MM, Kampinga HH et al: Post-irradiation dietary vitamin E does not affect the development of radiation-induced lung damage in rats. *Radiother Oncol*, 2004; 72: 67–70
24. Wu Z, Wang X, Yang R et al: Effects of carbon ion beam irradiation on lung injury and pulmonary fibrosis in mice. *Exp Ther Med*, 2013; 5: 771–76
- 15 25. Hamama S, Gilbert-Sirieix M, Vozenin MC et al: Radiation-induced enteropathy: molecular basis of pentoxifylline-vitamin E anti-fibrotic effect involved TGF- β 1 cascade inhibition. *Radiother Oncol*, 2012; 105: 305–12
26. Pan J, Su Y, Hou X et al: Protective effect of recombinant protein SOD-TAT on radiation-induced lung injury in mice. *Life Sci*, 2012; 91(3–4): 89–93
27. Riley PA: Free radicals in biology: oxidative stress and the effects of ionizing radiation. *Int J Radiat Biol*, 1994; 65: 27–33
- 20 28. Hemmati AA, Nazari Z, Ranjbari N et al: Comparison of the preventive effect of vitamin C and E on hexavalent chromium induced pulmonary fibrosis in rat. *Inflammopharmacology*, 2008; 16: 195–97
29. Seifirad S, Keshavarz A, TAclimi S et al: Effect of pirfenidone on pulmonary fibrosis due to paraquat poisoning in rats. *Clin Toxicol (Phila)*, 2012; 50: 754–58
- 25 30. Harrison SA, Torgerson S, Hayashi P et al: Vitamin E and vitamin C treatment improves fibrosis in patients with nonalcoholic steatohepatitis. *Am J Gastroenterol*, 2003; 98: 2485–90
- 30 31. [Empty entry]
- 35 32. [Empty entry]
- 40 33. [Empty entry]
- 45 34. [Empty entry]
- 50 35. [Empty entry]
- 53 36. [Empty entry]