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Malignite-Lung and Pleural Malignancies

The Effect of Erdosteine on Irradiation-induced Lung Inflammation in Rats

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Abstract

Background: The study is designed to investigate the protective effect of erdosteine on the inflammation of rat lung exposed to radiation. **Methods:** Fifty rats were divided in five groups according to their treatment. Group A consisted of 12 rats with no treatment, 6 rats in Group B were treated with ether only, 10 rats in Group C were treated with erdosteine only, 10 rats in Group D were treated with radiation only and 10 rats in Group E were treated with erdosteine and radiation. The follow-up period was 60 days for all rats and the rats in Groups C and E are treated with erdosteine 600mg per day and rats in Groups D and E received 20 Gy radiation in one fraction under ether anesthesia on the 30th day of the study. On the 60th day of the study all rats were sacrified and both lungs are taken out en-bloc. The bronchoalveolar lavage and histological examination were performed on all rats. Pulmonary collapse, alveolar distension, oedema, congestion, bronchopneumonia, vascular hyperplasia and lymphoid hyperplasia were investigated and graded from 0 to 4. **Results:** Two rats in group B and one rat in group C were lost during the follow-up period. The cytological examination of the BAL fluid showed no difference in collected cells in groups A,B and C but, neutrophil and lymphocyte counts were found in groups D and E as 53%; 6% and 30%; 22%, respectively ($p<0.05$). In the histological examination all parameters in group E were found lower than those in group D but, this difference did not reach a significant level. **Conclusion:** It has been found that erdosteine has diminished the neutrophil accumulation in the lungs following radiation but, this effect has not been observed in the histological analysis because of limited number of rats and limited period of time.

Keywords: Radiotherapy, lung inflammation, erdosteine, rat

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INTRODUCTION

Thoracic irradiation, as an important treatment method to increase survival and to relieve symptoms, has been utilized in patients with advanced or somehow inoperable pulmonary cancers [1]. However, pathologies due to lung toxicity give rise to significant problems such as pneumonia or fibrosis secondary to radiation. These problems are known to be the most significant dose restricting complications of radiotherapy [2-5]. Among toxicities secondary to radiation, pulmonary fibrosis particularly has significance, as it is a permanent and irreversible process [5].

In a study including 94 patients who received radiotherapy, it was reported that 36% of the patients developed mild, and 13% developed severe radiation pneumonitis and that 3-year survival in patients developing mild and severe radiation pneumonitis were 38.2% and 0%, respectively [6]. Oxidative stress and also free oxygen radicals play an important role on the development of these radiotherapy-related complications [7].

Numerous agents have been used to prevent or treat radiation fibrosis, however, a safe and effective drug for preventing fibrotic complications of irradiation has not been reported [5]. For this purpose, antiinflammatory agents such as steroids and other drugs including pentoxifylline, alpha tocopherol, interferons, superoxide dismutase, amifostine, and N-acetyl cysteine have been used in experimentally as well as in clinical studies [4, 7- 12].

It is reported that N-acetyl cysteine may inhibit free oxygen radicals and may have a radioprotective effect on patients receiving radiotherapy [7]. Another homocysteine derivative erdosteine is known to have protective role on the release of free oxygen radicals beside to its mucolytic and mucomodulator properties [13-18]. We aimed to evaluate irradiation induced cellular influx in airways and possible protective effect of erdosteine in this injury in experimentally studied rat lungs.

MATERIALS AND METHODS

Experimental Animals

In this study, 50 adult male Swiss albino rats weighing between 240 and 280 grams were used. Manipulations of the rats were performed under the rules of the Institutional Animal Ethics Committee of Ege University, Izmir, Turkey, complying with the European Communities Council Directive (86/609/EEC) and guided by the "International Guiding Principles for Biomedical Research Involving

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Table 1. Histopathologic results

	Group A (fed)	Group B (Ether)	Group C (Erdosteine)	Group D (RT)	Group E (Erdost+RT)
Collapse	1.25	0.33	1.67	1.7	1.1
Alveolar distension	0.33	0	0	0	0
Edema	0.17	0	0.67	1	0.6
Congestion	1	1.33	1.78	1.6	1.2
Bronchopneumonia	0	0	1.22	2.7	2.2
Vascular thickening	0.33	0	0.56	1.9	0
Lymphoid hyperplasia	1.5	2	0.67	0	0

$p>0.05$

Animals" developed by the Council for International Organizations of Medical Sciences (NIH).

Study Groups

*A. **Control group:*** Rats being simply fed for two months and received no therapy (n=12).

*B. **Ether group:*** Rats being fed, received no therapy but put to sleep with using ether (n=8).

*C. **Erdosteine group***: Rats being fed and also given erdosteine (n=10).

*D. **Radiotherapy group:*** Rats being fed and received radiation (n=10).

*E. **Radiation+Erdosteine group:*** Rats being fed and exposed to radiation when under erdosteine therapy (n=10).

Groups A, B, C, and D were accepted as control groups while group E was taken as the therapy group.

Feeding and Therapy

The rats were kept in rooms with a temperature of 22-24°C in temperature, and all the animals were fed twice daily. The rats in groups C and E were given erdosteine raw-material via a nasogastric tube 300 mg twice daily, 600 mg in total following a faint ether application. Besides, a group of rats (group B) were merely on faint ether administration twice daily in order to detect the possible effects of ether on lungs and also to prevent this from affecting the results of this study. The rats were followed to find out whether they vomited after feeding. All the rats were fed for two months and received their therapies designed for their groups. Erdosteine therapy was initiated (zero day) one month before irradiation was started and on the 30th day, rats in groups D and E were exposed to a single dose [2, 19, 20], 20 Gy [2, 19] radiation posteroanteriorly, involving both lungs via utilizing a 6 MV photon irradiator, at a 90% isodose reference range, in a linear accelerator. In groups C and E, erdosteine therapy was continued for an additional 30 days following irradiation.

Histopathologic Evaluation

At the end of the 60th day of the study, all the experimental animals underwent median sternotomy under aseptic conditions following ether anesthesia. Following sternotomy, all the rats were sacrificed immediately after their tracheobranchial tree and both lungs were extirpated as a whole through a tracheal incision below the laryngeal level. Left lungs of the rats were resected at the left main bronchus level, and then fixated with a 10% formalin solution. In addition, bronchoalveolar lavage (BAL) was performed on their right lungs via application of 5 mL of 0.9% NaCl solution in a plastic feeding tube, which was proceeded through the tracheal incision to be placed at the right main bronchus level. The BAL specimens obtained were sent to the laboratory of pathology preserved in an icebag. The department of pathology carried out the examinations on rat lungs being oblivious of the therapy conducted. BAL fluids were centrifuged at a rate of 600 cycles/min, and then two of the specimens were fixated in methyl alcohol, while the other two in ethyl alcohol. As the preparations fixed with methyl alcohol were stained with Giemsa dye, hematoxylin-eosin dye was used to stain the preparations fixed with ethyl alcohol, and the percentage of each cellular component were given by counting 1000 cells from different areas in each preparation. Microscopically, findings including pulmonary collapse, alveolar distension, edema, congestion, bronchopneumonia, vascular thickening, and lymphoid hyperplasia were detected. These findings were scored between 0 and 4 (Score 0:-, Score 1:+/- , Score 2:+, Score 3:++, Score 4: +++). In BAL (lung washing) fluid, 300 cells were counted, and the results were specified in percentages.

Statistical Evaluation

The results were assessed by using Kruskal-Wallis and Mann-Whitney U tests, and p values below 0.05 were accepted as being significantly different.

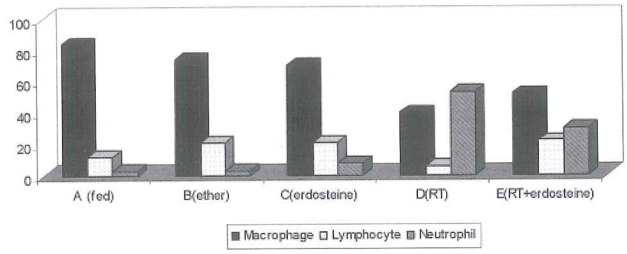

Figure 1. BAL results. When neutrophil ratios were assessed, it was found that levels in groups D and E were significantly higher than that of the remaining 3 groups, and in addition, when a comparison was made solely between groups D and E, it was also found that group E displayed a significant reduction in neutrophil ratio ($p<0.05$).

RESULTS

In our study, 3 rats died in total, 2 rats from group B, and one rat from group C, and it was determined that one of the rats from group B died on the 28th day of the study, the other one on the 42 th day, and the one from group C on the 26 th day of the study. All the deaths were observed to have resulted from the rats' attacking one another.

The ratio of alveolar macrophages in BAL examination was 83.9% (±9.7) in group A, 74.2% (±9.2) in group B, 70 % (±12.3) in group C, 40.1% (±8.4) in group D, and 51.8% (±8.2) in group E, and it was found that no significant difference existed between the groups ($p>0.05$). When lymphocyte ratios were examined; it was 12 % (±2.9) in group A, 21.2% (±4.4) in groupB, 21.4% (±4.7) in group C, 5.8% (±2.3) in group D, 22% (±4.6) in group E, and it was detected that ratio of lymphocytes in group D was significantly lower than those in the other groups ($p<0.05$). Neutrophil ratios were 3.1% (±1.8) in group A, 2.9% (±1.5) in group B, 8% (±2.4) in group C, 53.3% (±5.4) in group D, and 30% (±3.3) in group E. When neutrophil ratios were assessed, it was found that levels in groups D and E were significantly higher than that of the remaining 3 groups. In addition, when a comparison was made solely between groups D and E, it was also found that group E displayed a significant reduction in neutrophil ratio ($p<0.05$). The results of BAL examinations are given in Figure 1.

All the groups were analyzed for the findings which may pathologically occur in lungs including alveolar distension, collapse, edema, congestion, bronchopneumonia, vascular thickening, and lymphoid hyperplasia, and it was found that all the parameters in group E revealed lower results than they did in group D, but statistically there was no significant difference between the two groups ($p>0.05$). Histopathologic assessment results are given in Table 1.

DISCUSSION

Owing to the fact that oxidative stress and free oxygen radicals are shown to be effective in development of radiotherapy-induced inflammation and pulmonary fibrosis and that there are published studies indicating the inhibitory effect of erdosteine on the release of free oxygen radicals [13-18], this experimental study was designed with a consideration that erdosteine might play a role in the prevention of pulmonary toxicity secondary to radiation.

No data denoting the effects of erdosteine in radiation-induced pulmonary toxicity was available in the literature. Nonetheless, in a study by Maasilta et al., it was reported that 10 patients with non-small cell lung cancer were given N-acetyl cysteine via inhalation before radiotherapy, and given an additional dose intravenously and concurrently with radiotherapy, and it was found to be an expensive therapeutic option at the end of the one year follow-up [7]. Furthermore, data indicating that amifostin shows radioprotective effect owing to its antioxidant properties is striking. [4, 21]. Antanodou et al. compared 32 patients receiving only radiochemotherapy with 36 patients receiving 300 mg/m^2 amifostin in addition to radiochemotherapy and they found that acute pulmonary toxicity was 19.4% in amifostin group versus 56.3% in the other group. They reported that amifostin significantly reduced early and late pulmonary toxicity secondary to radiotherapy [4].

It is reported that a single dose of 20-36 Gy RT is sufficient to produce radiation-induced pulmonary toxicity such as radiation pneumonitis and pulmonary fibrosis [19, 20, 22], for this reason single dose of 20 Gy RT was applied in our study. However pulmonary toxicity of radiotherapy especially can arise near to 36 Gy RT we applied 20 Gy RT. For that reason our results may not reach significant level.

Five different groups of rats were used in our experimental study. A group was designed to observe the effects of erdosteine alone on lungs, and in addition, as ether would be used prior to erdosteine administration, an ether group was constituted to observe the potential effects of ether, and also to evaluate its effects on study results. At the end of the study, when these two groups were compared with the group solely fed and received no therapy, it was observed that no difference existed in cell distribution of BAL, and also in histopathologic findings between the groups. Thus, it may be considered that ether has no effect on study results, and similarly erdosteine has no effect on cellular distribution of BAL fluid, or on histopathologic evaluation.

In our study, neutrophil ratios in groups D and E were detected to be higher than those in the other groups, and this was considered to be due to the inflammatory effect of

radiation, as both groups were given radiotherapy. When group D (just receiving radiotherapy) and group E (receiving erdosteine in addition to radiotherapy) were assessed reciprocally, neutrophil ratio of BAL in group E was found lower than in group D. Hence, it can be noted that the reduction in neutrophilic alveolitis arising from the effect of radiotherapy is due to erdosteine. It is reported that neutrophils play a role on the secretion of mediators playing roles in various cellular activities such as superoxide anions, glutathione peroxidase, catalase, hydrogen peroxide, and nitric oxide, and also on the release of free oxygen radicals, it is also reported that erdosteine is in vivo and in vitro effective in reducing both neutrophil count and the levels of free oxygen radicals releasing from the neutrophils [17, 23-29]. In their in vitro study, Hosoe et al. reported that levels of H_2O_2 decreased due to the effect of the sulfhydryl group in M1 metabolite of erdosteine, but also denoted that this effect emerged in higher doses [30]. Hayashi et al. demonstrated that neutrophilic lung inflammation and acute lung injury in mice generated by lipopolysaccharides and hypochlorous acid administration could be prevented by giving erdosteine [31], and similarly Dal Sasso et al. also demonstrated that the sulfhydryl group, as a constituent of erdosteine, played an important role in inhibiting the release of free oxygen radicals from neutrophils [23]. In our study, a reduction in neutrophil count was demonstrated with the use of erdosteine, and it was also regarded that free oxygen radicals could be indirectly reduced in the same way. However, an important shortcoming of our study was that the levels of free oxygen radicals were not measured.

In our study erdostein did not influence cell differentiation of BAL and histological structure of parenchyma in group C. We know that erdostein can prevent leucocyte recruitment and improve mucocliary clearance especially under the inflammatory conditions [26, 31, 32]. In group C because of there were no any inflammatory state we didn't show any effect of erdostein

When the groups were compared in terms of pulmonary collapse, alveolar distension, edema, congestion, bronchopneumonia, vascular thickening, and lymphoid hyperplasia no significant difference was observed, and although it was statistically insignificant, it was determined that the parameters of the group receiving erdosteine concurrently with RT were lower than those of the group receiving RT alone. Nishioka et al. demonstrated the presence of fibroproliferative changes in rats on histopathologic examination which was performed after 8 weeks of a single dose of 30 Gy RT aplication [20]. Due to the fact that histopathologic evaluation was performed 4 weeks after RT, we assume that the absence of significant histopathologic differences might be a consequence of the inadequate time interval. Radiation pneumonitis and pulmonary fibrosis can appear approximately one to three and 6 months after RT respectively [22, 33]. We also know that risk of radiation pneumonitis can reach at top level at 3rd months of RT. However we finished our study at the end of 1st month because of technical problems. Although radiation induced cytokine abnormalities can be seen at the early period of RT we did not investigate cytokine levels.

In conclusion, it was demonstrated that RT-induced alveolitis occurring in rats could be diminished by using erdosteine however, that this effect did not produce histopathologic changes, which might presumably be due to the inadequacy of the number of rats, in addition to the shortness of the study period. Further experimental and human studies are necessary in order to unveil the role of erdosteine in preventing RT-induced pulmonary toxicity as a significant problem.

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