**ORIGINAL ARTICLE**

# Effects of hormonotherapy administered after concurrent radiotherapy and Trastuzumab on pulmonary fibrosis

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**Abstract**

**Aim:** This study was to investigate whether the use of hormonotherapy after concurrent radiotherapy (RT) and trastuzumab (T) has a contribution to the development of radiation fibrosis in the lungs.

**Materials and Methods:** Seventy Wistar Albino rats were divided into seven groups as follows: Group C: control, Group RT: RT only; Group T: trastuzumab only; Group RT+T+Tam: tamoxifen following concurrent RT and trastuzumab; Group RT+T+Le: letrozole following concurrent RT and trastuzumab; Group RT+T+An: anastrazole following concurrent RT and trastuzumab; Group RT+T+Exe: exemestane following concurrent RT and trastuzumab. Trastuzumab was prepared at an equivalent dose of 6 mg/kg. RT was administered 2 hours after T to the thoracic region at a dose of 12 Gy. Hormonotherapy was initiated one week after RT and administered by oral gavage once daily for 6 months. At the end of 24 weeks, the rats were sacrificed after being sedated with anesthesia. Both lungs were removed en bloc and blocked in paraffin. The level of fibrosis in each cross-section was assessed with the help of a scale.

**Results:** Significant differences were observed between the groups in terms of pulmonary fibrosis scoring. Statistically significant differences were observed when the radiotherapy group was compared to the C, T, T+RT+An, T+RT+Le and T+RT+Exe groups ($p<0.05$). Significant differences were found between the T+RT+Tam group and the C, T, T+RT+An, T+RT+Le and T+RT+Exe groups ($p<0.05$).

**Conclusion:** This study showed that the sequential administration of aromatase inhibitors following concurrent thoracic irradiation and T decreases radiation-induced pulmonary fibrosis. However, tamoxifen was found to have an opposite effect. Hippokratia 2013; 17 (3): 228-232

**Keywords:** Pulmonary fibrosis, hormonotherapy, trastuzumab, irradiation, rats

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## Introduction

Breast cancer is the most common type of cancer in women. Since it is an heterogeneous disease, treatment is planned based on the factors of the individual patient and the tumour. Adjuvant chemotherapy, radiotherapy and endocrine treatments provide significant decreases in breast cancer recurrence and mortality rates[1-2]. However, overexpression of human epidermal growth factor receptor 2 (HER-2) protein or HER-2 gene amplification occurs in approximately 15-25% of patients. This is accompanied by an aggressive behavior of the tumour[3-4].

In the recent years, attention has focused on HER-2/neu protein-targeted monoclonal antibody treatment in the management of breast cancer. Trastuzumab, monoantibody developed against HER-2 molecule, is used in the adjuvant treatment of HER-2/neu-positive breast cancer as a standard treatment[3-5]. The concurrent or sequential use of the drug with radiotherapy during adjuvant use has become an essential method. However, the possible late side effects that may be caused by this concurrent or sequential use are still not completely known[3-6].

During breast irradiation, lung tissue is also affected. Therefore, the lung is one of the most sensitive organs for observing the late effects of radiation. The radiation-induced damage in the lung is classified into two major groups. The early-stage damage is radiation pneumonia and the late-stage damage is pulmonary fibrosis[7].

It is not clearly known whether alone trastuzumab causes fibrosis in the lung tissue. The hormonotherapy drug tamoxifen is known to increase pulmonary fibrosis. Although there are insufficient data about aromatase inhibitors, there is information suggesting that they do not

increase fibrosis[3,4,8-10].

The aim of this experimental study is to investigate how the sequential use of hormonotherapy after concurrent trastuzumab with radiotherapy affects pulmonary radiation fibrosis by using pathological parameters.

**Materials and Methods**

This experimental study was received permission from the Animal Experiments Local Ethics Committee of Erciyes University Medical School.

*Experimental animals and study groups*

Wistar Albino rats aged 8 weeks and weighing 213 ± 27 grams were used in the study. The rats were divided into 7 groups with 10 animals in each group. The groups were:

Group C: The control group. No treatment was administered.

Group RT: The radiotherapy-only group. The thoracic region was irradiated while the animals were under anesthesia. Starting one week later, distilled water was administered by oral gavage once daily for six months.

Group T: The T-only group. T was administered via the tail vein. One week later, distilled water was administered as described for group RT.

Group T+RT+Tam: The group that was given tamoxifen after concurrent radiotherapy and T.

Group T+RT+Le: The group that was given letrozole after concurrent radiotherapy and T.

Group T+RT+An: The group that was given anastrozole after concurrent radiotherapy and T.

Group T+RT+Exe: The group that was given exemestane after concurrent radiotherapy and T.

In the T+RT+Tam, T+RT+Le, T+RT+An and T+RT+Exe groups, the rats were firstly administered trastuzumab via the tail vein. Two hours later, thoracic irradiation, covering both lungs, was performed. The assigned hormonotherapy drugs were crushed and dissolved in distilled water and administered orally once daily for six months.

*Trastuzumab administration*

The trastuzumab (Herceptin, Roche, Turkey) used in the study was administered at a dose of 6 mg/kg, which is accepted as the standard maintenance dose for 3-week courses in humans. Trastuzumab was administered via the tail veins of the rats after reconstitution with 100 cc saline solution and the calculation of the equivalent dose based on the weight of each rat.

*Radiotherapy administration*

The whole thoracic region was irradiated within 2 hours of the trastuzumab administration. The rats were planned for radiotherapy in groups of four under ketamine anesthesia. They were placed on a Plexiglas plate in supine position. The forelegs and hind legs of the rats were fixed. An irradiation area that covers both lungs was determined. RT was applied to a 5x30 cm area in a single fraction and at a dose of 12 Gy to the chest wall region, using a Co-60 device (Theratron 780 C, Canada).

*Hormonotherapy Administration*

As the half-life of T was reported to be 1 week in studies conducted in animals, hormonotherapy was administered one week after T. Equivalent doses of tamoxifen (20 mg/60 kg; Tamoksifen-Teva tablet, Med-İlaç, Turkey), anastrozole (1 mg/60 kg; Arimidex film tablet, AstraZeneca, Turkey), letrozole (2.5 mg/60 kg; Femera film tablet, Novartis, Turkey) and exemestane (25 mg/60 kg, Aromasin tablet, Pfizer, Turkey) were calculated for rats with a mean weight of 200 gr. The drugs were crushed and then dissolved in distilled water. Then, the drugs were given.

*Tissue sampling*

At the end of the study, the animals were sacrificed after being sedated with anesthesia. Both lungs were fixed with 10% formaldehyde administered via trachea. The lung tissues were removed en bloc. They were washed in isotonic saline solution. The samples were placed in formalin for histopathological examination.

*Histopathological examination*

Samples were taken from various regions of both lungs that were fixed in formalin. Following paraffin blocking procedure, serial cross-sections were obtained. They were stained with hematoxylin-eosin. Mean fibrosis scoring was obtained for the lungs of each rat. The scoring system for the intensity of fibrosis is as follows:

Score 0: No fibrosis or minimal fibrosis in the alveolar/bronchial wall; 1: moderate fibrosis that does not cause marked structural damage in the lungs; 2: increased fibrosis with definite damage to lung structure and formation of fibrous bands or small fibrous masses; 3: fibrosis that causes severe distortion in the lung structure and that has large fibrous areas; 4: total fibrosis.

*Statistical Analysis*

SPSS 13.0 (Statistical Package for Social Sciences for Windows, SPSS Inc., Chicago, IL) and SigmaStat 3.5 statistical package software, were used for the analyses. The normality tests for the data were performed with Shapiro-Wilks analysis. The comparison of more than two qualitative variables was done using Kruskal-Wallis test. The multiple comparisons were done using Student-Newman-Keuls method. $p< 0.05$ was accepted statistically significant.

**Results**

No deaths occurred in the groups during the study. The median values obtained from the assessment of pulmonary fibrosis scoring are presented in Table 1.

Statistically significant differences were found between the radiotherapy group and the control, T, T+RT+An, T+RT+Le, and T+RT+Exe groups. The radiotherapy group had an increased score when compared to

**Table 1:** Individual scores in the lungs

| Groups | Score values Mean | Score values Median (25-75%) | p (<0.05) |
|---|---|---|---|
| **Group C** | 0.0 | 0.0-0.0 | *** |
| **Group RT** | $1.0^{13567}$ | 1.0-3.0 | *** |
| **Group T** | 0.0 | 0.0-0.0 | *** |
| **Group T+RT+Tam** | $2.0^{13567}$ | 1.0-3.0 | *** |
| **Group T+RT+Le** | 0.0 | 0.0-1.0 | *** |
| **Group T+RT+An** | 0.0 | 0.0-0.0 | *** |
| **Group T+RT+Exe** | 0.0 | 0.0-1.0 | *** |

The groups in the same column with different numbers are statistically significant ($p<0.05$).

the control group ($p<0.05$). Thus, it was found that radiation causes fibrosis in the lungs. The RT group had a higher total fibrosis score when compared to the T+RT+An, T+RT+Le and T+RT+Exe groups. It was found that An, Le and Exe do not increase fibrosis in the lungs. Furthermore, anastrazole provided a larger decrease in pulmonary fibrosis although the difference between the group given T+RT+An and the RT T+RT+Le ve T+RT+Exe groups was not statistically significant. When the RT group was compared to the T+RT+Tam group, radiotherapy was found to increase the total score, although this increase was not statistically significant.

When the T-only group was compared to the control group, the values for the pulmonary fibrosis scoring were similar. Thus, the administration of trastuzumab alone did not cause pulmonary fibrosis. Statistically significant differences were found between the T+RT+Tam group and the control, T, T+RT+An, T+RT+Le and T+RT+Exe groups ($p<0.05$).

The pulmonary tissue samples of the experimental groups were histopathologically examined, the control, T and T+RT+An groups had no fibrosis (Figure 1a), while the T+RT+Exe and T+RT+Le groups had miminal fibrosis (Figure 1b). In the RT group, pulmonary fibrosis was moderate to marked (Figure 1c). In the T+RT+Tam group, moderate-to-severe pulmonary fibrosis was observed in the lung structure (Figure 1d).

## Discussion

Due to the success of mammography in early diagnosis and the advances in chemotherapy and radiotherapy, breast cancer is the type of cancer with most frequently changing treatment and it has many treatment combinations. After surgery, patients are initially treated with chemotherapy and then receive RT. It is still unclear whether hormonotherapy should be used sequentially or concurrently with RT. Hormonotherapy is generally initiated after RT[3,4,8,9].

In our literature review, we could not find any experimental or clinical studies demonstrating the late side effects of sequential hormonotherapy given after concurrent RT and T treatment. It is not known whether T and hormonotherapy cause additional toxicity compared to the expected side effects of RT when hormonotherapy is administered after concurrent or sequential use of T with RT. In our study, radiation-induced pulmonary fibrosis developed in the RT-only group. In the T-only group, no development of fibrosis was observed and the fibrosis score was the same as that in the control group. Except for tamoxifen, hormonotherapy given sequentially after concurrent administration of RT and T did cause an increase in radiation fibrosis. Trastuzumab, which is currently very commonly used in the treatment of breast cancer, was found to have radiosensitizing effect in both experimental and clinical studies. Pietras et al reported a significant radiosensibilization of combined T and radiation exposure compared with T or radiation alone in an experimental model of MCF-7 cell lines overexpressing HER-2 and in xenograft tumors[11]. Koukourakis et al, investigated the synergistic antitumor effect of T in combination with RT in HER-2-positive high-risk and chemoresistant breast cancer[12]. Belkamine et al, reported the

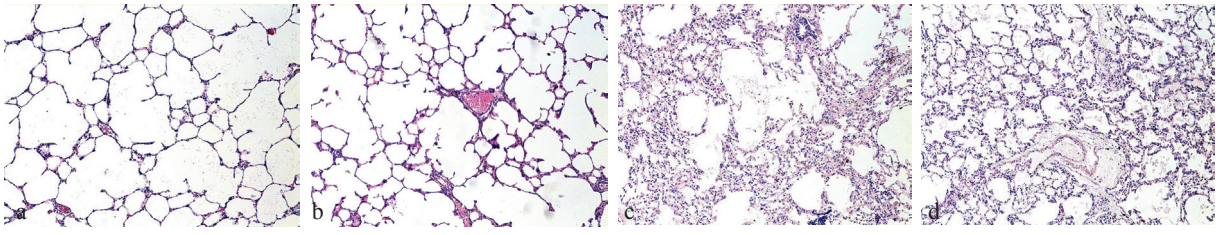


**Figure 1:** The histopathological images in all groups: 1a) the control, T and T+RT+Ana groups had no fibrosis, 1b) the T+RT+Exe and T+RT+Le groups had miminal fibrosis, 1c) in the RT group, pulmonary fibrosis was moderate, 1d) in the T+RT+Tam group, moderate-to-severe pulmonary fibrosis was observed (H&E, x200).

potential synergistic effect on concurrent T-RT on normal tissue involved in the radiation field[6].

In other studies, a "radioprotector" effect of trastuzumab was identified. In their study in rats, Bese et al. reported that the addition of trastuzumab to thoracic irradiation either sequentially or concomitantly did not increase radiation-induced pulmonary fibrosis[13].

Since the adjuvant use of trastuzumab is new in clinical practice, available data on the use of T with RT and the side effects are limited. In a study by Raben et al., the acute toxicities in breast and chest wall were compared in breast cancer patients who were treated with external RT with or without concomitant T. No significant difference was noted between both groups in terms of fatigue, edema, breast pain, and skin reactions[14]. Horton et al., in a phase I/II study investigating the combined use of T and RT in patients with locally advanced and recurrent breast cancer refractory to chemotherapy, gave only RT to patients with cardiac problems or with tumors over-expressing HER-2. The total survival at the end of the study was 35 months in the T+RT group versus 29 months in the primary RT group and the difference was not statistically significant. In conclusion, it was emphasized that administration of T concurrently with RT does cause an increase in side effects[15]. Another study, Perez et al. reported a retrospective comparison of irradiated versus nonirradiated patients from the North Central Cancer Group Phase III trial. After an 18-month median follow-up, concurrent T and RT administration did not increase skin toxicity, interstistial pneumonitis and dyspnea. In conclusion, the studies showed that the sequential or concurrent use of T with RT does not cause an increase in side effects. The results showing the late side effects are not available yet[4].

Randomized studies showed that, endocrine treatment should be given after chemotherapy in patients receiving both endocrine treatment and chemotherapy. Although selected studies showed that breast cancers that over-produce the HER-2 gene may be relatively refractory to hormonotherapy, other studies have not confirmed this[2,8,9,16].

It is not known whether T and hormonotherapy cause additional toxicity compared to the expected side effects of RT when hormonotherapy is administered after concurrent or sequential use of T with RT. Beşe et al, investigated the fibrosis stages by using tamoxifen sequentially or concurrently with RT in rats. They found that the incidence of radiation fibrosis was 3% in the RT-only group, 10% in the RT+Tam group and 36% in the concurrent RT+Tam group ($p<0.05$)[10]. Koç et al, in a clinical study conducted in patients with breast cancer, reported that the rate of pulmonary fibrosis was 13% in the group given only RT and 35% in the group given tamoxifen concurrently with RT ($p<0.05$)[17]. In the randomized phase 3 CONSET trial, patients undergoing mastectomy or breast-conserving surgery for tumors over 5 cm were enrolled. Patients were evaluated to 5 years of treatment. The rates of pulmonary fibrosis with concurrent and sequential administration were 30% and 15%, respectively. In conclusion, they reported that sequential treatment should be advised if RT will be administered to a patient receiving tamoxifen and especially if the peripheral lymphatic region will be included in the RT region[16]. Consistent with the literature, in our study, fibrosis developed in the group that was administered RT. When the RT group was compared with the RT+T+Tam group, it was found that there was a non-significant increase in radiation-induced fibrosis in both groups, while the increase was higher in the RT+T+Tam group. It was seen that tamoxifen increased the fibrosis.

In our study, there was no increase, and infact a decrease in the pulmonary fibrosis scores of the RT+T+Le, RT+T+An and RT+T+Exe groups when compared to the RT group. In our study, aromatase inhibitors were seen to be radioprotective. Among this group, anastrozole provided the largest decrease in fibrosis, although the decrease was not statistically significant. In their study, Azria et al. compared patients receiving letrozole sequentially or concurrently with RT. In the concomitant group, letrozole was started 3 weeks before RT, whereas it was initiated 3 weeks after RT in the sequential group. A three-dimensional conformal RT treatment (50 Gy and 6-18 MV) was administered. At the evaluation performed 24 months after the end of the treatment, no difference was noted between both groups. In conclusion, they reported that letrozole could be safely administered concurrently with RT, even with peripheral lymphatic irradiation[18]. In their study where they administered anastrozole sequentially or concurrently with RT in postmenopausal breast cancer patients with equal co-morbidities and similar tumor sizes and hormone receptor rates, Valakh et al. reported that the concurrent use did not increase the acute or late complication rates[9].

## Conclusion

When the results of our study were evaluated with respect to pulmonary fibrosis, it was seen that the use of T alone did not cause an excess toxicity. The sequential administration of aromatase inhibitors following the administration of trastuzumab concurrently with RT caused no excess toxicity with respect to side effects, compared to the effect of RT. In contrast, it was seen to reduce radiation fibrosis. However, it was seen that tamoxifen was associated with an increase in radiation fibrosis.

## Conflict of interest

There is no conflict of interest.

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