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Original article

Aromatase inhibitors decrease radiation-induced lung fibrosis: Results of an experimental study

A.Y. Altinok [a, *], S. Yildirim [b, 1], T. Altug [c, †], N. Sut [d, 2], A. Ober [e, 3], E.M. Ozsahin [f, 4], D. Azria [g, 5], N.S. Bese [e, 3]

[a] Department of Radiation Oncology, Medipol University, Medical School, TEM Avrupa Otoyolu Goztepe cikisi, No:1 Bagcilar, Istanbul, Turkey
[b] Department of Pathology, GATA Haydarpasa Training Hospital Selimiye Mh., Tibbiye Cd., 34668, Uskudar, Istanbul, Turkey
[c] Department of Experimental Animal Breeding and Research Laboratory, Cerrahpasa Medical School, Istanbul University, Turkey
[d] Department of Biostatistics, Trakya University, Medical School Balkan Yerleskesi, 22030, Edirne, Turkey
[e] Department of Radiation Oncology Cerrahpasa Medical School, Istanbul University Cerrah Pasa Mh., Cerrahpasa Tıp Fk., No:53, 34098 Fatih/Istanbul, Istanbul, Turkey
[f] Department of Radiation Oncology, CHUV,CH-1011, Lausanne Rue du Bugnon 46, 1011, Lausanne, Switzerland
[g] Department of Radiation Oncology, CRLC Val d'Aurelle, 31, Rue Croix Verte, 34000, Montpellier, France

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ABSTRACT

Purpose: In experimental and clinical trials, tamoxifen (TAM) has been shown to increase radiation-induced lung fibrosis (RILF). Furthermore, aromatase inhibitors (AI) have been shown to be superior to TAM in the adjuvant setting and preclinical data suggest that letrozole (LET) sensitizes breast cancer cells to ionizing radiation in other studies. In this experimental study, we evaluated whether AI have any impact on the development of RILF in rats.

Materials and methods: 60 female wistar- albino rats were divided into 6 groups: Control (group A), RT alone (group B), RT + TAM (group C), RT + anastrozole (ANA group D), RT + LET (group E), and RT + exemestane (EXE, group F). RT consisted of 30 Gy in 10 fractions to both lungs with an anterior field at 2 cm depth. Equivalent doses for 60 kg adult dose per day of TAM, ANA, LET, and EXE were calculated according to the mean weight of rats and orally administrated with a feeding tube. Percentage of lung with fibrosis was quantified with image analysis of histological sections of the lung. The mean score values were calculated for each group. the significance of the differences among groups were calculated using one way ANOVA test and Tukey HSD post-hoc test.

Results: Mean values of fibrosis were 1.7, 5.9, 6.7, 2.5, 2 and 2.2 for groups A, B, C, D, E, and F, respectively ($p = 0.000$). TAM increased RT-induced lung fibrosis but without statistical significance. Groups treated with RT + AI showed significantly less lung fibrosis than groups treated with RT alone or RT + TAM ($p = 0.000$). RT + AI groups showed nearly similar RT-induced lung fibrosis than control group.

Conclusions: In this study, we found that AI decreased RT-induced lung fibrosis to the control group level suggesting protective effect.

Introduction

In large randomized studies, third generation Aromatase Inhibitors (AI) have been shown to be superior to Tamoxifen (TAM) in the adjuvant systemic therapy of postmenopausal women with endocrine responsive early breast cancer [1,2] and upfront AI have been recommended as a part of standard treatment in this patient population. Postoperative whole-breast irradiation is an essential component of breast conserving surgery and post mastectomy radiation therapy has been demonstrated to drastically reduce

* Corresponding author. Tel.: +90 212 4607866; fax: +90 212 4607070.
E-mail addresses: ayseyildirimaltinok@gmail.com (A.Y. Altinok), syildirim@gata.edu.tr (S. Yildirim), nsut@trakya.edu.tr (N. Sut), aober@superonline.com (A. Ober), mahmut.ozsahin@chuv.ch (E.M. Ozsahin), David.Azria@valdorel.fnclcc.fr (D. Azria), nuranbese@superonline.com (N.S. Bese).
[1] Tel.: +90 212 444 1898; fax: +90 212 4441890.
[2] Tel.: +90 284 23576411632; fax: +90 284 2357630.
[3] Tel.: +90 0212 414 3000; fax: +90 2126320050.
[4] Tel.: +41 21 3141111; fax: +41 21 3141110.
[5] Tel.: +33 04 67 61 85 21; fax: +33 04 67 61 23 15.
[†] Deceased.

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locoregional recurrences and improve overall survival in high-risk patients [3–5].

The integration of these two common treatment modalities and their use in clinical practice concurrently or sequentially is not well established or known in detail. Insufficient data is available regarding the toxicity of concurrent use of AI and radiation therapy (RT). However in a preclinical study it has been shown that an AI, Letrozole (LET) may have a sensitizing effect on breast cancer cells to ionizing radiation which may lead to increased toxicity when used concurrently with RT in clinical setting [6]. Therefore, in this experimental study, we aimed to study the late effects of concurrent use of AI with irradiation and we evaluated weather AI have any impact on the development of radiation-induced lung fibrosis (RILF) in rats. Lung is chosen as an end organ as it is one of the most radiosensitive tissues to evaluate for late effects of RT [7]. We tested different molecules of AI both steroidal (Exemestane; EXE) and non-steroidal inhibitors (Anastrozole; (ANA) and LET) which interact with the aromatase enzyme differently. In addition we also retested the impact of nonsteroidal antiestrogen TAM with concurrent irradiation which has been shown to increase RILF [8].

Materials and methods

Sixty female Wistar albino rats, weighting approximately 200 g each were used in this study. Animals were bred, raised and housed in the Experimental Animal Breeding and Research Laboratory in X Medical School. Ten animals were housed per cage and maintained under identical conditions with food and water provided ad libitum. All experiments were carried out in compliance with the regulations of our institution and the 3R (reduction, replacement, refinement) ethical guidelines and ethical approval was obtained from the local Experimental Animal Research Ethical Committee. Wistar albino rats were randomized into 6 experimental groups and number of rats per group was 10. The first group of rats were the control group that was kept without receiving any treatment. (Group A). The second group had irradiation to whole thoracic region (Group B). The third group received TAM (Group C), the fourth group had ANA (Group D), the fifth group had LET (Group E) and the sixth group received EXE (Group F) in addition to thoracic irradiation (Table 1).

All five groups, excluding group A were irradiated to the whole thoracic region with Cobalt 60 unit at the Radiation Oncology Department of X Medical School. Whole lungs of the rats were simulated and marked prior to irradiation (Fig. 1). Animals were anesthetized with an intramuscular (IM) injection of Ketamine-HCL at a dose of 50 mg/kg, prior to simulation and irradiation. Animals were held securely on a foam holder in a supine position and plastic bandages were used to immobilize the thoracic region during irradiation. Irradiation was fractionated to analyze the effect of hormonal treatment with concomitant administration. A total dose of 30 Gy in 10 fractions which has been shown to cause RILF in rats was administrated [8] in 5 fractions per week to a 4 × 4 cm anterior single field at 2 cm depth.

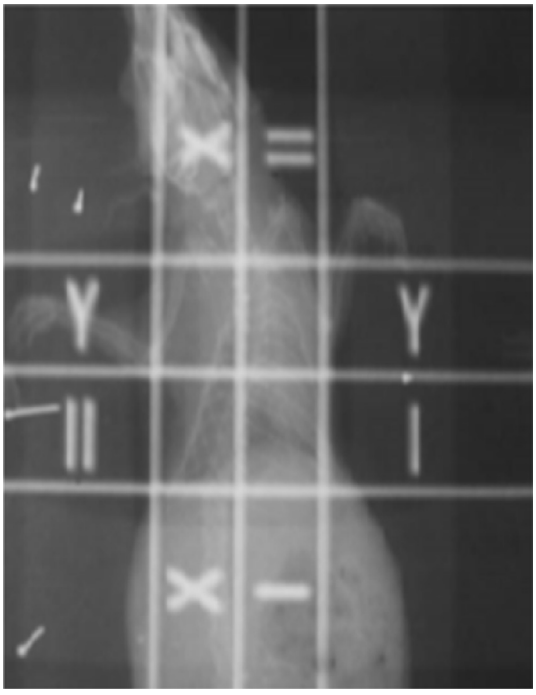

Fig. 1. Simulation of irradiated zone.

Standard dosage of hormonotherapy for adults was correlated to rats on weight basis. Average adult was presumed to be 60 kg and the average weight for subject rats was 200 g. The results of calculations is summarized in Table 2. Equivalent doses for 60 kg adult dose per day of TAM (clinical Nolvadex 10 mg tablet; gift of AstraZeneca pharmaceutical company), ANA (clinical Arimidex 1 mg gift of Astra-Zeneca pharmaceutical company), LET (clinical Femara 2.5 mg gift of Novartis pharmaceutical company) and EXE (clinical Aromasin 25 mg gift of Pfizer pharmaceutical company) were calculated according to the mean weight of rats which was 200 gr and orally administrated with a feeding tube. Administration was started at the first day of RT and continued with a daily single dose, including the week-ends, until the animals were sacrificed. Animals were anesthetized and sacrificed with cervical dislocation 16 weeks after RT which was shown to be a sufficient period for the development of RILF in rats [9]. Both lungs were removed and fixed by tracheal instillation of 10% neutral-buffered formalin and then embedded in paraffin. Four micron thickness of tissue sections were obtained and stained with Masson's Trichrome to observe lung fibrosis which is a late effect of RT. Fibrosis was defined as the thickened alveolar walls with superimposed collagen. As quantitative end point, the area of fibrosis in the alveolar walls was scored by a pathologist blinded to experimental groups, using an image analyzer (an IBM-Pentium II computer and Samba-400 IPS program (Software)) attached to a stereomicroscope on a scale of 0 (normal lung or minimal fibrous thickening) (Fig. 2) to 4 (total fibrous obliteration of the field) (Fig. 3) as described in Table 3.

Table 1
The distribution of animals according to the study groups are shown.

Groups	Description
Group A (n = 10)	Control
Group B (n = 10)	Irradiated group- (10 × 300 cGy)
Group C (n = 10)	Irradiated + Tamoxifen
Group D (n = 10)	Irradiated + Anastrozole
Group E (n = 10)	Irradiated + Letrozole
Group F (n = 10)	Irradiated + Exemestane

Table 2
Drug dosage.

Drug	Adult human dosage (60 kg)	Rat dosage (200 g)
Tamoxifen	20 mg	0.067 mg
Anastrozole	1 mg	0.003 mg
Letrozole	2.5 mg	0.008 mg
Exemestane	25 mg	0.083 mg