Concomitant trastuzumab with thoracic radiotherapy: a morphological and functional study

G. Yavas1, F. Yıldız1*, S. Guler2, M. F. Sargon3, D. Yıldız1, T. Yolcu1, M. Tuncer2 & F. H. Akyol1

1Departments of Radiation Oncology; 2Departments of Pharmacology; 3Departments of Anatomy, Hacettepe University Faculty of Medicine, Ankara, Turkey

Received 13 July 2010; revised 21 August 2010; accepted 23 August 2010

Background: The purpose of this study is to elucidate if there is an additive or supra-additive toxic effects of radiotherapy (RT) and trastuzumab (T) on vascular structures when used concomitantly.

Methods: Female Wistar albino rats were treated with either 8 or 15 Gy of thoracic RT. T was applied i.p. with a dose of 6 mg/kg 2 h before RT. Four rats in each arm were killed at 6th h, 21st and 70th days after irradiation and thoracic aorta of each animal was dissected for electron microscopy. In addition, functional studies for evaluating the relaxation and contraction were carried out 21 days after RT.

Results: Only 15-Gy RT dose groups showed significant difference in terms of functional deterioration as more contraction than the others (P < 0.05) without any difference between RT and RT + T. However, T produced additional deficit in relaxation when added to RT, which was considered near significant (P: 0.0502). Electron microscopy showed endothelial and subendotelial damage signs in 15-Gy dose groups. T + 15-Gy arm showed more pronounced endothelial cell damage than 15-Gy RT-only arm, 70 days after RT.

Conclusion: T and high-dose RT may lead to vascular damage that seems at least additive.

Key words: radiotherapy, trastuzumab, vascular damage

introduction

Radiation-induced changes in blood vessels have been known since Gassmann [1] described the histological picture of X-ray damage in small blood vessels of the skin. Numerous studies of radiation injury show that the injury of endothelial cells is the key point in most tissues that ultimately leads to fibrosis or necrosis [2–7]. The sequence of endothelial injury, cell detachment, thrombosis and fibrosis results in significant tissue injury that often limits radiation oncologist in attempting to deliver curative doses to a nearby tumor. The functional parameters relating to capillary endothelium is permeability, blood flow and perfusion pressure. Edema, as a result of increased permeability, may occur within hours of radiation. Further injury results in detachment of the basement membrane and formation of the microthrombi, which leads to further reduction in blood flow [7]. The decrease of blood flow becomes maximally visible at 3 weeks of radiation.

It was shown by Fajardo and Steward [8–10] that acute inflammation occurred ~6 h after radiotherapy (RT). A latent phase with a slight progressive fibrosis begins ~2 days after exposure. Electron microscopy demonstrates a progressive damage leading to obstruction of the lumen and thrombi of fibrin and platelets. Though healthy endothelial cell replication in the vicinity occurs, it is generally inadequate and an inevitable ischemia leads to progressive fibrosis. Animals begin to die at ~70th day due to extensive fibrosis.

Fifteen percent to 25% of breast cancers express human epidermal growth factor receptor 2 (HER2) amplification [11]. Trastuzumab (T) is a recombinant DNA-derived monoclonal antibody that selectively binds to the extracellular domain of the HER2 protein in breast cancer cells [12, 13]. Five randomized controlled trials have addressed the addition of adjuvant T to adjuvant chemotherapy in node-positive and high-risk node-negative patients with HER2 overexpression and showed a survival advantage with T [14–19].

T has been shown to produce cardiac dysfunction in metastatic and early breast cancer patients [20–22]. RT on the other hand leads to not only pancarditis but also vascular damage. Especially, patients with breast cancer who are applied internal mammary irradiation are candidates for vascular damage. To our knowledge, there is no published data regarding the vascular toxicity of RT and T when used concomitantly. In this experimental study, we aimed to evaluate whether there is additive or supra-additive effect of T and RT on vascular structures.

methods and materials

study design

Seventy-two female Wistar albino rats 250–300 g were used in the study. (Permission no.: 2009/37-5 of Experimental Animals Ethical Committee of Hacettepe University). Rats were divided into six groups (G) composed of...
12 animals. In G1, rats were sham irradiated. G2 was defined as T control group. G3 and G5 were the RT-only arms and single doses of either 8 Gy (G3) or 15 Gy (G5) of RT was administered, respectively. G4 and G6 were the RT and T groups, respectively, in which 8 Gy (G4) or 15 Gy (G6) dose of RT concomitant with T was applied (Table 1).

Four rats in each arm were killed at 6th h, 21st and 70th days after irradiation and thoracic aorta of each animal was dissected for electron microscopy. In addition, functional studies for evaluating the relaxation and contraction of aortic ring-like segments mounted in isolated organ bath were carried out 21 days after RT.

**Irradiation protocol**

RT was applied under general anesthesia with i.p. administered 90 mg/kg ketamine hydrochloride and 10 mg/kg xylazine. A single dose of 8 or 15 Gy with 6-MV photon beams was applied via a single anterior field to 2-cm depth with SAD technique. The field size was 4 × 4 cm and included the whole thoracic aorta.

**Trastuzumab protocol**

T dose which was equivalent to 6 mg/kg adult dose was calculated for each rat and injected i.p. 2 h before the RT. The rats in G1, G3 and G5 were applied 0.5 cc 0.9% NaCl i.p.

**Preparation of thoracic aorta rings and measurement of pharmacological responses**

On the 21st day, the rats were anesthetized with 90 mg/kg ketamine hydrochloride and thoracotomy procedure was carried out. The thoracic aorta was dissected and 5-mm rings were prepared. Two parallel wires were placed in the lumen of the rings and the tissues were mounted to a 20-ml organ bath filled with physiological saline solution (in mmol/l: NaCl, 118; KCl, 4.6; NaHCO3, 25; MgSO4, 1.2; KH2PO4, 1.2; CaCl2, 1.2; glucose, 10 and EDTA, 0.025) and aerated with 95% O2 and 5% CO2 gas mixture. The responses to ACh were reported as the concentration. As the plateau was reached, acetylcholine (ACh) was applied in increasing concentrations. The responses to ACh were reported as the percentage of the potassium contraction responses were taken in the increasing concentrations of alcohol series and the tissues were washed with propylene oxide and embedded in epoxy resin embedding media. The semi-thin and ultrathin sections of the obtained tissue blocks were cut with an ultramicrotome (LKB Nova, Bromma, Sweden). These semi-thin sections that were 2 μm in thickness were stained with methylene blue and examined under a light microscope (Nikon, Tokyo, Japan). Then, trimming was done and their ultrathin sections which were ~60 nm in thickness were taken by the same ultramicrotome. These ultrathin sections were stained with uranyl acetate and lead citrate and they were examined under Jeol JEM 1200 EX (Japan) transmission electron microscope.

**Statistical analysis**

Two-way analysis of variance test for functional evaluation was used to calculate the significance of the differences among groups. Tests were bilateral and a P value of <0.05 was considered significant. For electron microscopy, thoracic aorta samples of each group were studied and differences were registered. The parameters which were investigated by electron microscopy for each rat were endothelial cells, subendothelial layer, internal elastic membrane, smooth muscle cells of the tunica media, external elastic membrane and collagen fibers and fibroblasts of tunica externa (adventitia).

Since only morphological appearance of endothelial cells and the severity of subendothelial edema were different among groups, a scoring system, similar to Emir et al. [24], was carried out based on these two parameters (Table 2).

**Results**

**Functional results**

The functional studies of aorta on the 21st day after RT revealed subtle endothelial dysfunction. Contractions with 90 mM KCl did not differ among groups. Only 15-Gy RT dose groups showed significant difference in PE contraction (P < 0.05). There was no significant difference between 15-Gy-only and 15-Gy + T groups. Although 8-Gy RT dose groups showed slightly more contraction than control groups, this did not reach significance (Figure 1). T did not cause an extra contraction deficit.

There was no significant difference among groups regarding relaxation responses mediated by ACh. However, when groups were combined as RT (G3 + G5) versus RT + T (G4 + G6), T

**Table 1.** The abbreviations used for the study groups

<table>
<thead>
<tr>
<th>Group (G)</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>Sham-irradiated control group</td>
</tr>
<tr>
<td>G2</td>
<td>Trastuzumab control group</td>
</tr>
<tr>
<td>G3</td>
<td>8-Gy group</td>
</tr>
<tr>
<td>G4</td>
<td>8-Gy + trastuzumab group</td>
</tr>
<tr>
<td>G5</td>
<td>15-Gy group</td>
</tr>
<tr>
<td>G6</td>
<td>15-Gy + trastuzumab group</td>
</tr>
</tbody>
</table>

**Table 2.** The scoring system for the damage signs of vascular injury

<table>
<thead>
<tr>
<th>Endothelial cells:</th>
<th>Subendothelial edema</th>
</tr>
</thead>
<tbody>
<tr>
<td>0: normal</td>
<td>0: no edema</td>
</tr>
<tr>
<td>1: slight thinning</td>
<td>1: minimal edema</td>
</tr>
<tr>
<td>2: apparent thinning</td>
<td>2: intensive edema</td>
</tr>
</tbody>
</table>
produced a deterioration in the relaxation function, which was considered near significant ($P: 0.0502$) (Figure 2).

electron microscopy

There were no apparent changes detected ultrastructurally in control groups and 8-Gy dose groups 6 h after RT. However, 15-Gy dose groups either RT alone or RT + T showed early subendothelial and mitochondrial edema signs in addition to vacuolization in tunica intima. The severity of morphological damage was not different between the two groups.

Subendothelial edema scores of groups 5 and 6 were scored as $0.75 \pm 0.5$ without any major difference between individual rats in each group (Table 3).

Again, there were no apparent morphological changes in control groups and 8-Gy dose groups 21 days after RT. There was no morphological difference due to senescence. On contrary, 15-Gy dose groups showed prominent changes. Endothelial cells were found very thin and in some areas detachment from basement membrane was observed. There were huge vacuoles in tunica intima and intensive subendothelial edema was seen. Addition of T to 15 Gy RT revealed no significant deterioration (Figure 3). The morphological scores of each group were calculated as 0 for groups 1–4 regarding to endothelial cells and subendothelial edema. The corresponding scores for G5 and G6 were $2.25 \pm 0.5$ for endothelial and $1.75 \pm 0.5$ for subendothelial damage (Table 4).

Minimal subendothelial edema was observed in 8-Gy dose groups, 70 days after RT. Fifteen-Gray groups on the other hand showed morphological signs of severe damage. In G5, endothelial cells were obviously thinner and in some areas, they lost their cytoplasmic organelles. Detachment from basement membrane was obvious in some areas (Figure 4). T when added to 15 Gy dose of irradiation led to deterioration of morphological signs (Figure 5). The mean subendothelial edema scores of each group were $0$ for control groups, $0.75 \pm 0.5$ for 8-Gy dose groups, $1.75 \pm 0.5$ for 15-Gy RT and 2 for 15-Gy RT + T groups. The endothelial damage scores of control and 8-Gy RT groups were $0.75 \pm 0.58$ for G5 and 4 for G6 (Table 5). The total damage scores of G5 and G6 were calculated as 5.25 and 6, respectively.

discussion

In this study, we aimed to find out if there is an additive or supra-additive toxic effect of RT and T on vascular structures when used concomitantly and found that 15 Gy single dose of irradiation produced significant morphological and functional damage. T when combined with RT led to further relaxation deficit and augmentation of signs of morphological damage, which is evident 70 days after irradiation.

Rat models of cardiovascular pathology contribute toward understanding and treatment of a broad range of conditions since they are relatively inexpensive and large sample size can be produced in a relatively short period of time. Though there are several limitations to use of rat models regarding differences in myocardial functions compared with human heart, rat aortic models are frequently used in studies examining chronic heart failure (CHF), hypertension, cardiomyopathy and ischemia [25]. Both aorta and coronary arteries are conduit vessels and
show similar properties. So it is reasonable to think that our findings in rat aorta model can also represent changes in coronary arteries.

Lots of studies which evaluate radiation injury on vascular structures revealed that endothelial cell damage is the key point [2–6, 8–10]. Fifteen Gray dose of irradiation in our study led to prominent endothelial and subendothelial changes in thoracic aorta which became worse as time went by. Eight Gray of RT on the other hand did not cause any apparent morphological and functional damage. In a study by Verbeke et al. [26], 0, 5, 10, 15, 20 and 25 Gy doses of RT were applied on rat aortic rings with cobalt-60 gamma-rays and it was found that at least 15 Gy dose of irradiation was needed to observe a functional defect. Similar studies revealed that the most severe morphological changes were seen in >20 Gy RT doses [26–30]. Endothelial cells’ response to lower doses of radiation can be obtained in the clinical manifestations but it is expected to be less [31]. The vast majority of our study was correlated with literature data. Eight Gray dose of RT was not enough to cause significant changes both from functional and morphological aspects.

Table 3. The semiquantitative scoring of endothelial cells and subendothelial edema, 6 h after radiotherapy

<table>
<thead>
<tr>
<th>Group</th>
<th>Endothelial (mean ± SD)</th>
<th>Subendothelial edema (mean ± SD)</th>
<th>Total score (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>0.75 ± 0.75</td>
<td>0.75 ± 0.5</td>
</tr>
<tr>
<td>6</td>
<td>0</td>
<td>0.75 ± 0.5</td>
<td>0.75 ± 0.5</td>
</tr>
</tbody>
</table>

SD, standard deviation.
The irradiation doses used in routine clinical practice are different from the ones used in animal studies. Most patients in routine practice are treated with conventional fractionation to a total dose of 50–70 Gy. However, in recent years, stereotactic radiosurgery and intraoperative RT that use single or two to five fractions of high-dose irradiation have become popular. It has been postulated that the linear-quadratic model is an appropriate methodology for determining isoeffective doses at large dose per fraction [32]. The 15 Gy single dose of RT in our study corresponds to 48–54 Gy, the most frequent dose range used in clinical practice, when α/β ratio of 3–4 is used.

Radiation-induced cardiovascular disease is observed especially in patients treated for Hodgkin’s lymphoma, breast cancer and lung cancer. Acute injury often seen as pericarditis is generally transient. However, late injury as CHF, coronary artery disease (CAD) manifests several months to years after treatment and can be life threatening. Long-term results of phase III trials of postmastectomy RT in breast cancer patients [33, 34] usually reveal an increased risk of cardiac mortality especially when left-sided and internal mammary nodal RT was used. Animal models suggest that radiation can cause both microvascular and macrovascular cardiac pathology [35]. Macrovascular injury accelerates age-related atherosclerosis and leads to CAD years after RT [36]. Microvascular injury on the other hand reduces capillary density within months after RT and decrease collateral flow. It has been known that myocytes are relatively radioresistant to direct cytotoxic effects of irradiation and radiation-induced heart damage is an effect of indirect myocyte toxicity secondary to vascular injury [35].

Data collected from Hodgkin’s disease patients revealed that anteriorly placed coronary arteries were more often affected by RT compared with circumflex artery [37]. The risk of death from CAD increases by 3% per every Gray increase in the total dose and the risk increase is higher for women irradiated at ages 20–49 than at older ages [38]. In our study, young adult rats weighing 250–300 g in weight were used and assumed to correspond to young adult female patients. The study lasted for 70 days and no morphological signs of aging could be detected in control groups. T is a recombinant DNA-derived monoclonal antibody that selectively binds to extracellular domain of the HER2 protein in breast cancer cells [15, 16, 39–41]. The pathophysiology of T-related cardiotoxicity lies on the beneficial effect of erbB2 on heart’s ability to respond to stress. It was shown that erbB2-deficient hearts were more susceptible to cardiotoxic effects of the stressor and the risk of irreversible loss of cardiac myocytes was increased. The reported incidence of cardiac injury in metastatic breast cancer patients is 2% when T is used alone, 13% when it is combined with paclitaxel and 27% when it is used with anthracyclines [21]. The data from NSABP B-31 and

Table 4. The semiquantitative scoring of endothelial cells and subendothelial edema, 21 days after radiotherapy

<table>
<thead>
<tr>
<th>Group</th>
<th>Endothelial (mean ± SD)</th>
<th>Subendothelial edema (mean ± SD)</th>
<th>Total score (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>2.25 ± 0.5</td>
<td>1.75 ± 0.5</td>
<td>4 ± 0.8164</td>
</tr>
<tr>
<td>6</td>
<td>2.25 ± 0.5</td>
<td>1.75 ± 0.5</td>
<td>4 ± 0</td>
</tr>
</tbody>
</table>

SD, standard deviation.

Table 5. The semiquantitative scoring of endothelial cells and subendothelial edema, 70 days after radiotherapy

<table>
<thead>
<tr>
<th>Group</th>
<th>Endothelial (mean ± SD)</th>
<th>Subendothelial edema (mean ± SD)</th>
<th>Total score (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>0.75 ± 0.5</td>
<td>0.75 ± 0.5</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>0.75 ± 0.5</td>
<td>0.75 ± 0.5</td>
</tr>
<tr>
<td>5</td>
<td>3.5 ± 0.5773</td>
<td>1.75 ± 0.5</td>
<td>5.25 ± 0.5</td>
</tr>
<tr>
<td>6</td>
<td>4 ± 0</td>
<td>2 ± 0</td>
<td>6 ± 0</td>
</tr>
</tbody>
</table>

SD, standard deviation.
potential interaction and do the treatment planning to severe vascular damage. The clinician should be aware of this even if it is not a clinical one, our experimental study revealed of ionizing radiation and T on vascular structures. However, there is no clinical data in the literature regarding the potential interaction especially when combined with anthracyclines. There is no concomitantly. T has been shown to be a cardiotoxic agent not only to dose, volume and technique of chest irradiation but thoracic aorta as well as CAD. Damage appears to be related to vascular toxicity. The lack of resistance to stress factors may lead erbB2-blocked myocardium to exaggerated signs of injury when combined with RT. There is no morphological data in the literature examining the cardiac toxicity when RT and T used together. The aim of our study was to evaluate the vascular toxicity and it was found that T did not produce any additional morphological damage at earlier times. However, it was found to augment the damage signs 70 days after RT. Though ours is a pure experimental study, we can get some clues to the clinics that T and RT combination can lead to more than additive damage to secondary damage to myocardium. The lack of evidence for the pathogenesis of radiation-induced injury to large arteries. J Laryngol Otol 1997; 111: 988–999.

As a conclusion, potential adverse effects of mediastinal irradiation are numerous and can include large arteries-like thoracic aorta as well as CAD. Damage appears to be related not only to dose, volume and technique of chest irradiation but also to the drugs, which are used either sequentially or concomitantly. T has been shown to be a cardiotoxic agent especially when combined with anthracyclines. There is no clinical data in the literature regarding the potential interaction of ionizing radiation and T on vascular structures. However, even it is not a clinical one, our experimental study revealed that mediastinal high-dose RT when combined with T may lead to severe vascular damage. The clinician should be aware of this potential interaction and do the treatment planning accordingly.

disclosure

The authors have declared no conflicts of interest.

references


