original article

Annals of Oncology 22: 1120–1126, 2011
doi:10.1093/annonc/mdq590
Published online 19 November 2010

Concomitant trastuzumab with thoracic radiotherapy: a morphological and functional study

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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

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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 10 mg/kg xylazine 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; $NaHCO_3$, 25; $MgSO_4$, 1.2; KH_2PO_4, 1.2; $CaCl_2$, 1.2; glucose, 10 and EDTA, 0.025) and aerated with 95% O_2 and 5% CO_2 gas mixture. Responses of the aorta rings were measured by a force displacement transducer and recorded by a polygraph (Grass Model 7B). Rings were rested for 30 min under a resting tension of 0.75 g.

Thoracic aorta segments were contracted with 90 mM KCl. The contraction responses were taken in the increasing concentrations of phenylephrine (PE) and were reported as the percentage of the potassium contraction. Then, aorta rings were precontracted with submaximal PE concentration. As the plateau was reached, acetylcholine (ACh) was applied at increasing concentrations. The responses to ACh were reported as the percentage of the submaximal PE contraction similar to Ïscher and Vanhoutte's [23].

electron microscopic examination

The tissue samples, which were taken from the rats of the third to the sixth groups, 6 h, 21 and 70 days after RT, were put into 2.5% glutaraldehyde for 24 h for primary fixation. The same application was done to the rats of the trastuzumab and control groups after the administration of the drug or NaCl in the same timing procedure. Then, these samples were washed with Sorenson's phosphate buffer solution (pH 7.4) and they were postfixed in 1% osmium tetroxide. After postfixation, they were washed with the same buffer and dehydrated in 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

Group (G)	
G1	Sham-irradiated control group
G2	Trastuzumab control group
G3	8-Gy group
G4	8-Gy + trastuzumab group
G5	15-Gy group
G6	15-Gy + trastuzumab group

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

Endothelial cells:
0: normal
1: slight thinning
2: apparent thinning
3: Detachment from basement membrane
4: Detachment from basement membrane and apparent thinning
Subendothelial edema
0: no edema
1: minimal edema
2: intensive edema

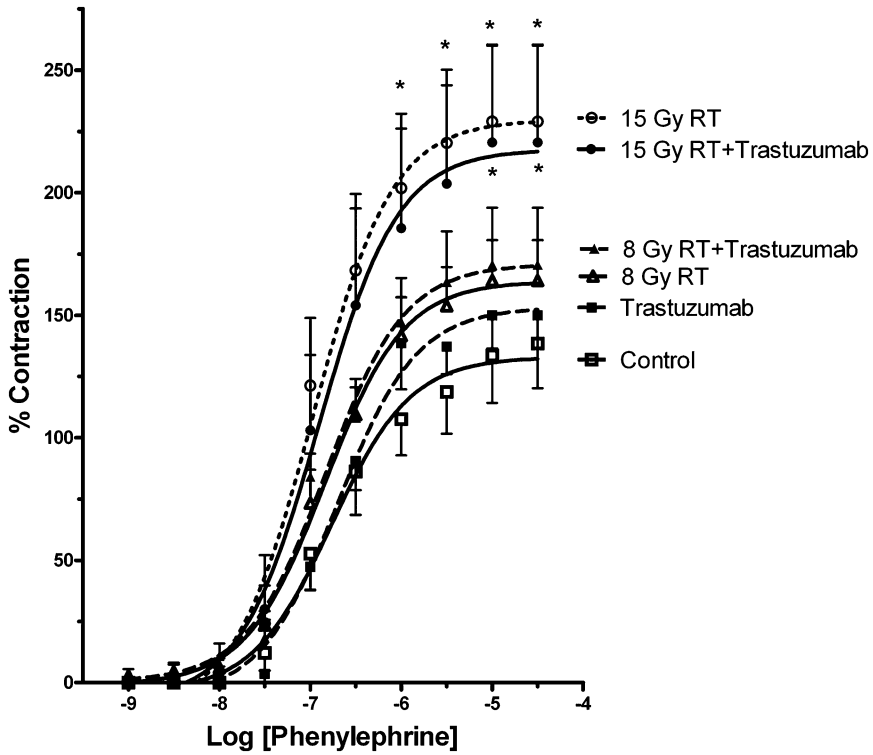

Figure 1. Contraction responses of groups on the 21st day after radiotherapy (RT). Contraction responses of groups. Only 15-Gy RT dose groups (G5 and G6) showed significant difference in terms of functional deterioration as more contraction than the others ($P < 0.05$)

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 ± 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 ± 0.5 for endothelial and 1.75 ± 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 ± 0.5 for 8-Gy dose groups, 1.75 ± 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, 3.5 ± 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

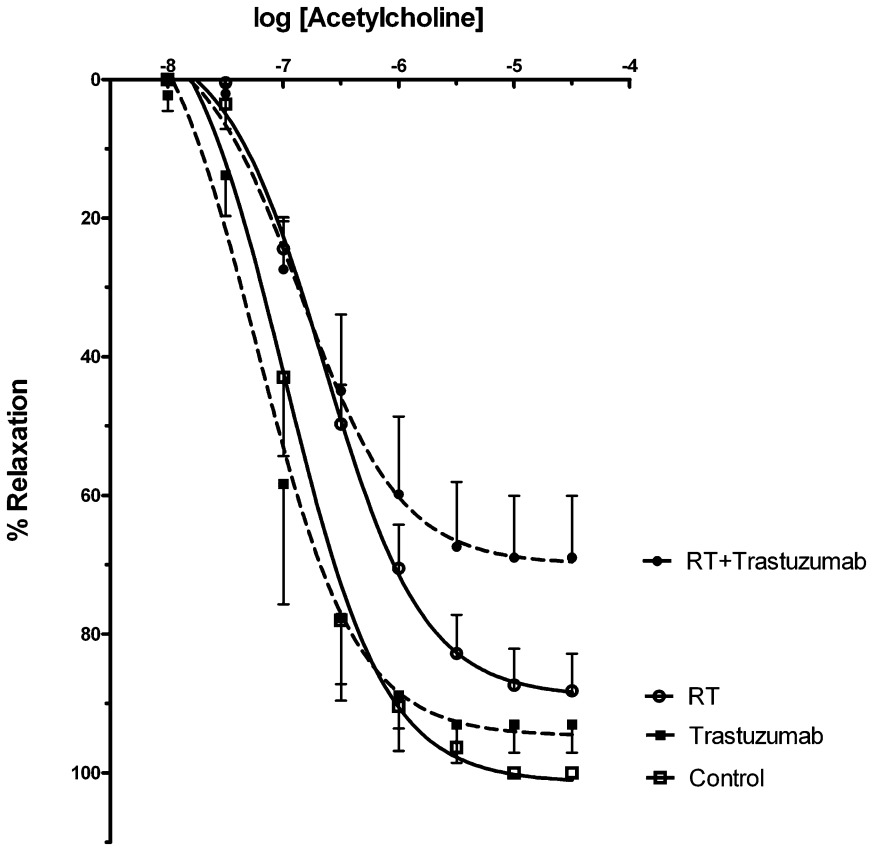

Figure 2. The relaxation function on the 21st day after radiotherapy (RT). Groups were combined as RT versus RT + T. RT + T showed less relaxation response, which was near at significant level (*P*: 0.0502)

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

Group	Endothelial (mean ± SD)	Subendothelial edema (mean ± SD)	Total score (mean ± SD)
1	0	0	0
2	0	0	0
3	0	0	0
4	0	0	0
5	0	0.75 ± 0.5	0.75 ± 0.5
6	0	0.75 ± 0.5	0.75 ± 0.5

SD, standard deviation.

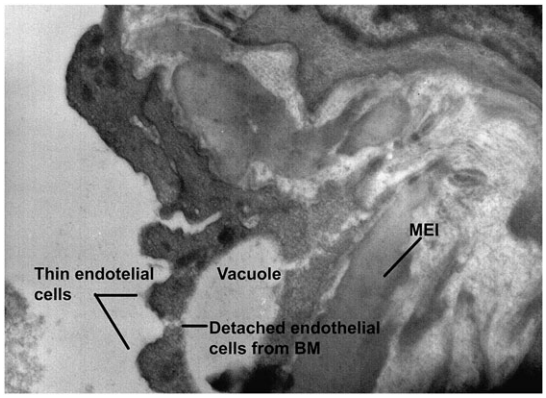

Figure 3. Electron microscopical appearance of 15-Gy radiotherapy + T group. Endothelial cells are thinner than normal cells. In some areas, detachment from basement membrane is observed. Vacuolization in tunica intima and obvious subendothelial edema is also seen. BM, basement membrane; MEI, membrana elastica interna.

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

Group	Endothelial (mean ± SD)	Subendothelial edema (mean ± SD)	Total score (mean ± SD)
1	0	0	0
2	0	0	0
3	0	0	0
4	0	0	0
5	2.25 ± 0.5	1.75 ± 0.5	4 ± 0.8164
6	2.25 ± 0.5	1.75 ± 0.5	4 ± 0

SD, standard deviation.

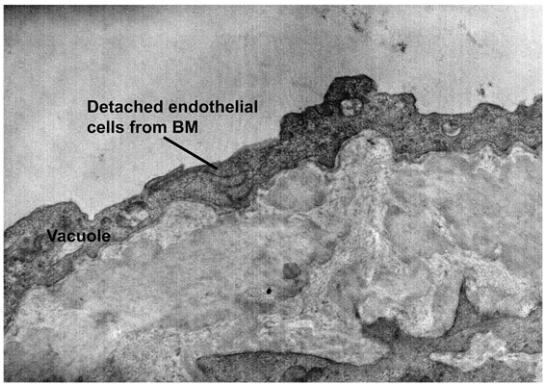

Figure 4. Electron microscopical appearance of 15-Gy radiotherapy group. Endothelial cells are obviously thin and in some areas, they lost their cytoplasmic organelles. Detachment from basement membranes in some areas is also seen. BM, basement membrane.

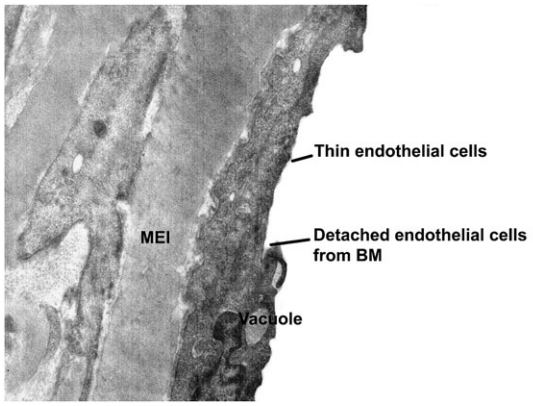

Figure 5. Electron microscopical appearance of 15-Gy radiotherapy + T group. Endothelial cells lost all of their cytoplasmic organelles and nuclei; they are as thin as a rope and they detached from the basement membrane (BM) in most areas. MEI, membrana elastica interna

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

Group	Endothelial (mean ± SD)	Subendothelial edema (mean ± SD)	Total score (mean ± SD)
1	0	0	0
2	0	0	0
3	0	0.75 ± 0.5	0.75 ± 0.5
4	0	0.75 ± 0.5	0.75 ± 0.5
5	3.5 ± 0.5773	1.75 ± 0.5	5.25 ± 0.5
6	4 ± 0	2 ± 0	6 ± 0

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 threading. 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

NCCTG N-9831 [15, 19] in early breast cancer revealed absolute risk of class III/IV CHF at 3 years as 4% and risk of asymptomatic and symptomatic cardiac dysfunction as 18.9% when T was used with chemotherapy.

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 [15, 16, 18, 19, 42] and found significant survival advantage with T. Halyard et al. [19] reported cardiac adverse event data from NCCTG 9831 trial. Concurrent adjuvant RT and T in this trial was not associated with increased cardiac complications. However, the median follow-up was only 3.7 years and internal mammary irradiation was not allowed in this trial. Similarly, in a retrospective study by Schaffer et al. [43], no deleterious effect of T on acute cardiac toxicity could be found when used with internal mammary irradiation. However, only data of 59 patients were included in this study in which only 13 of them were applied internal mammary irradiation and the mean follow-up time was only 15 months. It is well known that 3–20 years median time is needed to detect clinical radiation-induced cardiovascular toxicity in breast cancer patients [8]. RT does not produce direct cytotoxic effect on myocytes but radiation-induced vascular damage can lead to secondary damage to myocardium. 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 toxicity and the signs of this toxicity becomes apparent only after some time.

The presence of EGFR on myocardial cells has been shown in several studies; however, there is no study examining age-related expressions of EGFR on blood vessels. The major disadvantage of our study is that we do not know the EGFR content of aorta and cannot make a comment on how irradiation and T interact in terms of vascular toxicity. However, we are conducting a new study in which we investigate the EGFR availability on the thoracic aorta segments also.

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

1. Gassmann A. Zur Histologie der Röntgenulceria. Fortschr Geb Roentgenstr 1898; 2: 199–207.
2. Gold H. Production of arteriosclerosis in the rat: effect of x-ray and a high-fat diet. Arch Pathol Lab Med. 1961; 71: 268–273.
3. Lindsay S, Kohn HI, Dakin RL et al. Aortic arteriosclerosis in the dog after localized aortic x-irradiation. Circ Res 1962; 10: 51–60.
4. Lamberts HD, de Boer WGRM. Contributions to the study of immediate and early x-ray reactions with regard to chemoprotection: VII. X-ray-induced atheromatous lesions in the arterial wall of cholestrolemic rabbits. Int J Radiat Biol 1965; 9: 165–174.
5. Zidar N, Ferlugo D, Haula A et al. Contribution to the pathogenesis of radiation-induced injury to large arteries. J Laryngol Otol 1997; 111: 988–999.
6. Murros EK, Toole JF. The effect of radiation on carotid arteries: a review article. Arch Neurol 1989; 46: 449–455.
7. Rubin BD. The Radiation Biology of the Vascular Endothelium, 1st edition. CRC Press, Chapman and Hall 1998.
8. Adams MJ, Hardenbergh PH, Constine SL, Liphultz SE. Radiation-associated cardiovascular disease. Crit Rev Oncol Hematol 2003; 45: 55–75.
9. Stewart JR, Fajardo LF, Gilette SM, Constine SL. Radiation injury to the heart. Int J Radiat Oncol Biol Phys 1995; 31(5): 1205–1211.
10. Stewart JR, Fajardo LF. Radiation induced heart disease. Clinical and experimental aspects. Radiol Clin North Am 1971; 9: 511–531.
11. Benz CC, O'Hagen RC, Richter B et al. HER2/neu and the Ets transcription activator PEA3 are coordinately upregulated in human breast cancer. Oncogene 1997; 15: 1513–1525.
12. Wonders KY, Reigie BS. Trastuzumab and doxorubicin related cardiotoxicity and the cardioprotective role of exercise. Integr Cancer Ther 2009; 8: 17–21.
13. Albanell J, Bellmunt J, Moline R et al. Node negative breast cancers with p53 (-)/HER2-neu status may identify women with very good prognosis. Anticancer Res 1996; 16: 1027–1032.
14. Perez EA, Suman VJ, Davidson N et al. NCCTG N9831, May 2005 update Presented at the 45th annual meeting of the American Society of Clinical Oncology Orlando FL, May 16, 2005.
15. Romond EH, Perez EA, Bryan J et al. Trastuzumab plus adjuvant chemotherapy for operable HER-2 positive breast cancer. N Engl J Med 2005; 353: 1673–1684.
16. Smith I, Procter M, Gelber RD et al. 2-Year follow-up of trastuzumab after adjuvant chemotherapy in HER-2 positive breast cancer: a randomized controlled trial. Lancet 2007; 369: 29–36.
17. Slamon D, Eiermann W, Robert N et al. BCIRG 006: 2nd interim analysis phase III randomized trial comparing doxorubicin and cyclophosphamide followed by docetaxel (ACT) with doxorubicin and cyclophosphamide followed by docetaxel and trastuzumab (ACTH) with docetaxel, carboplatin and trastuzumab (TCH) in HER-2/nue positive early breast cancer patients. In Proceedings of the 29th Annual San Antonio Breast Cancer Symposium, San Antonio TX, December 14–17, 2006. Abstract.
18. Joensuu H, Kellokumpu-Lehtienen PL, Bono P et al. Adjuvant docetaxel or vineralbine with or without trastuzumab for breast cancer. N Engl J Med 2006; 354: 809–820.
19. Halyard MY, Pisansky TM, Dueck AC et al. Radiotherapy and adjuvant trastuzumab in operable breast cancer: tolerability and adverse event data from the NCCTG phase III trial N9831. J Clin Oncol 2009; 27(16): 2638–2644.
20. Vogel C, Cobleigh MA, Triphaty DA et al. Adjuvant docetaxel or vineralbine with or without trastuzumab as a single agent in first-line treatment of HER2-overexpressing metastatic breast cancer. J Clin Oncol 2002; 20: 719–726.

21. Slamon DJ, Leyland-Jones B, Shak S et al. Use of chemotherapy plus monoclonal antibody against HER 2 for metastatic breast cancer that overexpresses HER 2. N Engl J Med 2001; 344: 783–792.
22. Leyland Jones B, Gelmon K, Ayoub JP et al. Pharmacokinetics, safety and efficacy of trastuzumab administered every three weeks in combination with paclitaxel. J Clin Oncol 2003; 21: 3965–3971.
23. L̈scher TF, Vanhoutte PM. Endothelium-dependent contractions to acetylcholine in the aorta of the spontaneously hypertensive rat. Hypertension 1986; 8(4): 344–348.
24. Emir M, Gol MK, Ozisik K et al. Harvesting techniques affect the integrity of the radial artery: an electron microscopic evaluation. Ann Thorac Surg 2004; 78: 1319–1325.
25. Hasenfuss G. Animal models of human cardiovascular disease, heart failure and hypertrophy. Cardiovasc Res 1998; 39: 60–76.
26. Verbeke M, Thierens H, Toeymans Y, De Ridder L. An organotypical in vitro model for vascular tissue remodeling and its application to study radiation effects. Cytotechnology 2000; 34: 185–195.
27. Crocker IR. Endovascular gamma irradiation in the swine model: the Emory experience. In King SB, Walksman R, Crocker I (eds), Discoveries in Radiation for Restenosis, Abstract Book of Course at Lenox Atlanta, GA, USA 1996; 37–38.
28. Brenner DJ, Miller RC, Hall EJ. The radiobiology of intravascular irradiation. Int J Radiat Oncol Biol Phys 1999; 36: 805–810.
29. Serruys PW, Carlier SG. The scope of the problem of vascular restenosis. In Levandag PC (ed), Vascular Brachytherapy. New Perspectives. Remedica Publishing, London 1999; 4–7.
30. Shaffer U, Micke O, Dorszewki A et al. External beam irradiation inhibits neointimal hyperplasia after injury-induced arterial smooth muscle cell proliferation. Int J Radiat Oncol Biol Phys 1998; 42: 617–622.
31. Weinberger J, Simon AD. Intracoronary irradiation fort he prevention of restenosis. Curr Opin Cardiol 1997; 12: 468–474.
32. Brenner DJ. The linear quadratic model is an appropriate methodology for determining isoeffective doses at large doses per fraction. Semin Radiat Oncol 2008; 8: 234–239.
33. Ragaz J, Olivotto IA, Spinelli JJ et al. Locoregional radiation therapy in patients with high risk breast cancer receiving adjuvant chemotherapy: 20 year results of British Colombia randomized trial. J Natl Cancer Inst 2005; 97: 116–126.
34. Host H, Breenhovd I, Loeb M. Postoperative radiotherapy in breast cancer long term results from the Oslo study. Int J Radiat Oncol Biol Phys 1986; 12: 727–732.
35. Darby SC, Cutter DJ, Boerma M et al. Radiation related heart disease: current knowledge and future perspectives. Int J Radiat Oncol Biol Phys 2010; 76: 656–665.
36. Veinot JP, Edward WD. Pathology of radiation-induced heart disease: a surgical and autopsy study of 27 cases. Hum Pathol 1996; 8: 766–773.
37. Boivin JF, Hutchison GB, Lubin JH et al. Coronary artery disease mortality in patients treated for Hodgkin's disease. Cancer 1992; 69: 1241–1242.
38. Darby SC, McGale P, Taylor CW et al. Long-term mortality from heart disease ad lung cancer after radiotherapy for early breast cancer. Prospective cohort study of about 300000 women in US SEER cancer registries. Lancet Oncol 2005; 6: 557–565.
39. Hudis AC. Trastuzumab—mechanism of action and use in clinical practice. N Engl J Med 2007; 357: 39–51.
40. Yarden Y. The EGFR family and its ligands in human cancer: signaling mechanism and therapeutic opportunities. J Clin Oncol 2009; 18: 3471–3479.
41. Valabrega G, Montemurro F, Agiletta M. Trastuzumab mechanism of action, resistance and future perspectives in HER-2 overexpressing breast cancer. Ann Oncol 2007; 18: 977–984.
42. Slamon D, Eiermann W, Robert N et al. Phase III randomized trial comparing doxorubicin and cyclophosphamide followed by docetaxel (ACT) with doxorubicin and cyclophosphamide followed by docetaxel and trastuzumab (ACTH) with docetaxeli carboplatin and trastuzumab (TCH) in HER2 positive early breast cancer patients: BCIRG 006 study. Breast Cancer Res Treat 2005; 94: s5.
43. Shaffer R, Tyldesley S, Rolles M et al. Acute cardiotoxicity with concurrent trastuzumab and radiotherapy including internal mammary chain nodes: a retrospective single-institution study. Radiother Oncol 2009; 90: 122–126.