positivity rate was 50% in the patients, and 16% of the patients were 65 years of age or older. DFS was 81% in the TC group and 75% in the AC group ($P = 0.033$), and OS was 87% in the former and 82% in the latter ($P = 0.032$), showing that TC therapy was significantly superior regarding the two parameters. Concerning the safety profile, the incidence of febrile neutropenia (FN) was slightly higher in the TC than in the AC group, but long-term bone marrow toxicity and cardiotoxicity were low, showing that the therapy was feasible. It was also reported that the tolerability of elderly patients at 65 years of age or older was favorable, showing the superiority of TC therapy. Based on these, the standard post-operative adjuvant chemotherapy for early-stage breast cancer may change from the current regimens including anthracyclines to taxane-based regimens without anthracycline.

There are racial differences in the effects and adverse effects of chemotherapy. The standard regimens to administer many therapeutic drugs for breast cancer employed in Western countries are also applicable for Japanese, but the recommended doses of some drugs established by Phase II dose-setting studies conducted in Japan are lower than those in Western countries. The standard dose of DTX for every 3-week administration in monotherapy is 100 mg/m^2 in Western countries, but 70 mg/m^2 in Japan. Moreover, there is no safety data concerning the combination of DTX and CPA in Japan. To introduce the TC regimen (75/600) adopted in the USON9735 into Japan, the confirmation of its safety in Japanese is essential. Based on this background, we performed a study to confirm the safety of the TC regimen (75/600) of the USON9735.

PATIENTS AND METHODS

Of patients who underwent radical surgery for primary breast cancer at the Shikoku Cancer Center and Okayama University Hospital, those who met the following inclusion criteria were selected: an age between 20 and 70 years, and a risk category of intermediate or higher employing the 10th St Gallen risk classification assessed based on the clinical background and post-operative pathological diagnosis, i.e. hormone receptor-negative cases, lymph node metastasis-positive cases and lymph node metastasis-negative cases, hormone receptor-positive cases meeting one of the following conditions: a 2 cm or greater diameter of tissue invasion, histological grade of 2–3, 35 years of age or younger, the presence of severe vascular invasion and HER2-positivity. Estrogen (ER) and progesterone receptors were assessed by immunostaining, and a positive cell rate of 10% or higher was regarded as positive. HER2 was assessed by the Hercep test, and scores of 0 and 1+ were regarded as negative, and 3+ as positive. In cases graded 2+, the HER2/neu amplification rate was determined, and a 2.2 or higher rate was regarded as positive. Other inclusion criteria were the absence of distant metastasis and severe complications with a performance status of 0 or 1 and sufficient bone marrow, liver and renal functions. Written informed consent was obtained from all patients. Patients with pre-operative chemotherapy, a past medical history of drug allergy which may interfere with the therapy, inflammatory and bilateral breast cancers, double cancer and a past medical history of psychiatric diseases were excluded.

In the administration, after pre-treatment with 8 mg dexamethasone and 5-hydroxytryptamine $(HT)_3$ receptor blockade, 75 mg/m^2 DTX was administered by drip infusion for 60 min, followed by the administration of 600 mg/m^2 CPA for 30 min on day 1. From 12 h later, oral dexamethasone 4 mg was administrated twice daily for 2 days. These were administered every 21 days four times (four cycles). Blood testing was performed on the day of administration in each cycle to decide on the next administration. The criteria for initiating administration were as follows, and administration was postponed until all items recovered: WBC $\geq$3000/mm^2, neutrophil count $\geq$1500/mm^2, neuropathy $\leq$Grade (G) 2, edema $\leq$G2, liver dysfunction $\leq$G1 and renal dysfunction $\leq$G1. When these did not recover for 21 days from the scheduled administration day, the protocol was discontinued. When the following adverse reactions were noted in the previous cycle, the first dose reduction was performed based on the dose reduction criteria: (i) G3 or severer non-hematological toxicity, (ii) G4 or severer hematological toxicity excluding leukopenia and neutropenia and (iii) G4 leukopenia and neutropenia persisting for 7 days or longer. The level of first dose reduction was as follows: DTX, 60 mg/m^2 and CPA, 500 mg/m^2. When these adverse reactions were present after the dose reduction, the protocol was discontinued. Regarding supportive therapy, preventive antibiotic administration was prohibited, but administration for FN decided on by the attending physician was accepted. The preventive administration of granulocyte colony-stimulating factor (G-CSF) was also prohibited based on the ASCO 2006 guidelines, but according to the decision by the attending physician, the following administration criteria were accepted: (i) fever ($\geq$38°C) development with a neutrophil count of $<$1000/mm^2 or a neutrophil count of 500/mm^2 after the completion of drug administration, (ii) when an identical chemotherapy is employed after meeting the condition (i), the subsequent administration starts when the neutrophil count reaches 1000/mm^2.

The primary endpoint was set as the safety. The types and grades of adverse reactions were identified following the National Cancer Institute (NCI)—Common Terminology Criteria for Adverse Events (CTCAE) ver. 3.0, and the incidences of G3 or severer adverse reactions were evaluated. The secondary endpoint was set as the protocol treatment completion rate. During the protocol treatment period, the body weight and temperature were measured, and blood testing was performed to investigate adverse reactions once a week at the outpatient clinic. Expecting of the number of patients in our institutions for a year who correspond to inclusion criteria, the target number of enrollments was set

to 50. This study was approved by the Institutional Review Board.

The standard treatment arm in the USON9735 was AC (60/600), and the tolerability against this regimen has been reported and widely adopted in Japan. The dose of TC superior to AC shown by this trial should be accepted from a dose density viewpoint. Although a dose-setting study is necessary for the safety confirmation of translational combination therapy, the initial dose was set to TC (75/600). To ensure the safety of patients, an early stopping rule was established, in which enrollment was suspended after five early cases were enrolled until the completion of the protocol treatment in all five cases, and adverse reactions were evaluated. When the following adverse reactions were noted in two or more of the five cases, the study protocol was reviewed: (i) G3 or severer edema, (ii) G3 or severer peripheral neuropathy, (iii) FN, (iv) other G4 hematological toxicity and (iv) discontinuation of the protocol treatment due to an adverse reaction. The adverse event profiles of the five cases were submitted to the Effect/Safety Evaluation Committee to examine the feasibility of study continuation.

RESULTS

Enrollment was initiated in May 2007, and the five early cases were enrolled by July 2007. The protocol treatment was completed in four of the five cases, clearing the early stopping rule. Continuation of the study without protocol revision was approved by the Effect/Safety Evaluation Committee. Enrollment was re-started in September 2007, 53 were registered by October 2008 and the protocol treatment of the 53 cases was completed by January 2009. Adverse events could be adequately assessed in all patients.

The clinicopathological background factors of the patients are shown in Table 1. The median age was 54 years (33–67 years) and five patients (9.4%) were 65 years of age or older. Thirty-eight cases (71.7%) were ER-positive, 12 (22.6%) were HER2-positive, the mean tumor size was 1.94 cm (0.7–11.5 cm), and 22 cases (41.5%) were lymph node metastasis-positive, with a mean number of lymph node metastases of 1.4 (1–3).

The protocol treatment was completed in 50 of the 53 cases, with a completion rate of 94.3%. The protocol was discontinued in three due to fatigue, skin eruption, G4 leukopenia and neutropenia based on the judgment by the attending physician or patient's request. One was a 62-year-old female in whom G4 leukopenia and neutropenia occurred after the first cycle, and the dose was reduced following the dose reduction criteria in the second cycle, but her attending physician decided on discontinuation due to G4 hematological toxicity. The second case was a 67-year-old female in whom G4 leukopenia and neutropenia and G2 fatigue developed following the first cycle, and the treatment was discontinued based on the patient's request. The third case was a 61-year-old female in whom G4 leukopenia and neutropenia

Table 1. Patients characteristics

Category	*n*	%
Age		
<65	48	90.6
≥65	5	9.4
Median age	54 (33–67)	
Menopause		
Pre	24	45.3
Post	29	54.7
ER		
+	38	71.7
–	15	28.3
PgR		
+	32	60.4
–	21	39.6
HER2		
Positive	12	22.6
Negative	41	77.4
T		
T1	25	47.2
T2	27	50.9
T3	1	1.9
N		
0	31	58.5
1	22	41.5
Nuclear grade		
1	12	23.1
2	20	38.5
3	20	38.5
Number of pN		
0	31	58.5
1	16	30.2
2	3	5.7
3	3	5.7
Risk category		
Intermediate	49	92.5
High	4	7.5
Surgery		
Bp	32	60.4
Bt	21	39.6

ER, estrogen receptor; PgR, progesterone receptor; pN, pathological N; Bp, breast conserving; Bt, mastectomy.

Table 2. Hematologic toxicity

	Grade							
	1		2		3		4	
	n	%	*n*	%	*n*	%	*n*	%
Neutropenia	1	1.9	0	0	14	25	38	71.7
Leucopenia	0	0	5	9.4	30	56.7	17	32.1
Febrile neutropenia	0	0	0	0	15	28.3	0	0
Anemia	10	18.9	4	7.5	0	0	1	1.9

Table 3. Non-hematologic toxicity

	Grade					
	1		2		3	
	n	%	*n*	%	*n*	%
Fatigue	22	41.5	20	37.7	0	0
Aiopeicia	1	1.9	51	98.1	0	0
Arthralgia	18	34	2	3.8	0	0
Myalgia	18	34	3	5.7	0	0
Nausea	17	32.1	2	3.8	0	0
Vomiting	4	7.5	0	0	0	0
Constipation	11	20.8	2	3.8	0	0
Diarrhea	8	15.1	1	1.9	0	0
Edema	13	24.5	0	0	0	0
GOT, GPT	5	9.4	0	0	0	0
Nail change	15	28.3	0	0	0	0
Rash	18	34	10	18.9	1	1.9
Stomatitis	8	15.1	2	3.8	0	0
Watery eye	1	1.9	0	0	0	0
Neuropathy	10	18.9	2	3.8	0	0
Cystitis	2	3.8	0	0	0	0

GOT, glutamic oxaloacetic transaminase; GPT, glutamic pyruvic transaminase.

and G3 systemic skin eruption developed following the first cycle, and her attending physician decided on the discontinuation. Dose reduction conflicting with the dose reduction criteria was necessary in four cases (7.5%).

On hematological toxicity evaluation following the NCI-CTCAE, G3–4 leukopenia developed in 47 (88.7%), G3–4 neutropenia in 52 (98.1%) and FN in 15 (28.3%). G-CSF was administered to nine (17.0%). G4 anemia occurred in one (1.9%) (Table 2).

Regarding non-hematological toxicity, hair loss occurred in most patients, and G2 or milder fatigue in 42 (79.2%). Edema occurred in 13 (24.5%), but all were G1, and could be resolved by diuretic treatment. G2 or milder arthralgia and myalgia occurred in 20 (37.8%) and 21 (39.7%), respectively. Peripheral neuropathy developed in 12 (22.7%), but the severity was G2 or milder (Table 3).

As another non-hematological toxicity, skin eruption accompanied by pruritus appeared at a high incidence. In one case (1.9%), systemic skin eruption developed and was graded G3.

As subgroup analysis by age, the patients were divided into those aged 65 years or older and those younger than 65 years, as in the USON9735. Dose reduction was necessary in 2 of 48 patients younger than 65 years (4.2%), and 2 of 5 patients aged 65 years or older (40%). All patients in the younger group completed the protocol treatment, whereas only two of the five patients (40%) completed the treatment in the elderly group, with higher dose reduction rate, decreasing the completion rate. FN developed in 11 (22.9%) in the younger group and 4 (80%) in the elderly group, showing that the incidence of FN was also higher, and G-CSF support was more often needed in the elderly patients (Table 4).

Table 4. Summary by age group

	≥65 ($n = 5$)	<65 ($n = 48$)
Completion rate	40%	100%
Dose reduction	40%	4.2%
G-CSF support	40%	14.6%
Febrile neutropenia	80%	22.9%

G-CSF, granulocyte colony-stimulating factor.

DISCUSSION

Although the importance of systemic drug therapy to improve the prognosis of breast cancer is widely recognized, combination chemotherapy including anthracycline has been employed as the standard post-operative adjuvant chemotherapy after initial cyclophosphamide, methotrexate, 5-FU therapy (4–6). Many clinical studies have since been performed, and the efficacy of additional taxane administration for lymph node metastasis-positive and -negative high-risk cases was demonstrated, which attracted attention to taxanes for post-operative adjuvant therapy (7,8). Although the efficacy of anthracyclines was established, the development of cardiotoxicity and myelodysplasia as late adverse effects was often problematic. An increase in the incidence of cardiotoxicity to 4–18% in a cumulative dose-dependent manner has been reported (9). Three fatal cases due to heart disease and osteomyelodysplasia syndrome were also reported in the USON9735, for which the involvement of adriamycin could not be ruled out because of the administration of the AC arm alone (1). As HER2 and Topo IIα gene aberrations attract attention as anthracycline efficacy predicting factors, the

individualized administration of anthracycline in consideration of these predictive factors may also progress in the future (10,11). In addition, a recurrence-inhibitory effect of trastuzumab in HER2-overexpressing patients has been demonstrated, and the frequency of combining trastuzumab with cytotoxic drugs or consecutive administration has increased, with which the usefulness of taxanes with lower-level cardiotoxic adverse effects has been increasing (12,13). As the superiority of TC to AC therapy was demonstrated in the USON9735 (1,2), taxane regimens not including anthracycline may become a major trend in the future, and this trend may not be ignored in Japan. Regarding the safety, although the 9735 trial reported favorable tolerability (1), it is well known that there exists ethnic or racial difference in pharmacokinetics and pharmacodynamics. These have been attributed to the distinctions in the genetics, physiological and pathological factors. Moreover, these differences are also known to be influenced by several extrinsic factors such as socioeconomic backgrounds, culture, diet and environments (14,15). Therefore, the verification of tolerability and adverse effects is an important clinical task to introduce TC therapy into Japan. This is the first report on the safety of TC therapy in Japanese patients.

The overall completion rate was 94.3%, similar to that (93%) in the USON9735. The protocol treatment was discontinued in three cases (5.7%). Dose reduction was necessary in 7.5%. The dose intensities of DTX and CPA were 98.5% and 98.7%, respectively. The completion rate was mostly favorable, and fewer cases required dose reduction, but hematological toxicity: G3–4 leucopenia and neutropenia occurred in almost all cases. In the USON9735, the incidence of G3–4 neutropenia was 61%, slightly lower than that in the present study, but this difference may have been due to variation in the observation interval: every 3 weeks in the 9735 trial, whereas weekly in our study to closely observe adverse effects. Since the safety of TC therapy in Japanese was confirmed by this study, observation every 3 weeks and on the administration days may be sufficient for actual clinical practice. The incidence of FN in all cases was reported to be 5% in the USON9735, but attention should be paid to the fact that the administration of prophylactic antibiotics was accepted in the USON9735. No prophylactic administration was performed in our study, and the incidence was 28.3%. FN could be controlled by oral antibiotics in most cases, but G-CSF administration was necessary in 17%. Regarding hematological toxicity in AC therapy (60/600 mg/m^2), Tsutani et al. (16) reported that G3–4 neutropenia occurred in 24.3% and FN in 3.8% in Japanese. Based on these findings, the incidences of hematological toxicity and FN are apparently higher in TC than in AC therapy, to which closer attention should be paid. For actual clinical cases, prophylactic antibiotics administration may be considered. Regarding non-hematological toxicity, G2 or milder edema developed in 34% in the USON9735, whereas the grade was G1 or milder, and the incidence was only 24.5% in our study. Diuretics were administered to some cases, but most cases remitted under course observation alone. The incidences of nausea and vomiting were 35.9% and 7.5%, respectively, lower than those in the USON9735 (53% and 14%, respectively). Another non-hematological toxicity mentioned was skin eruption. The incidences of G1, G2 and G3 skin eruption were 34%, 18.9% and 1.9%, respectively, ~55% in total. Skin eruption persisted after the completion of four cycles in some cases. The establishment of effective countermeasures against skin eruption in TC therapy is necessary. Regarding DTX-induced skin eruption, although several cases have been reported, no therapy has been established, and only symptomatic therapy is available (17–19). The incidence in DTX monotherapy is reported to be 20–48%, suggesting that the combination with CPA increases the rate of development (20).

The subgroup analysis by age in the 9735 trial concluded that the incidence of adverse effects in elderly patients aged 65 years or older was not significantly different, and the tolerability of the elderly patients was favorable (1). In contrast, in our study, because of the small sample size, statistical comparison was not performed, the protocol treatment completion rate was lower in the patients aged 65 years or older than in those younger than 65 years (40% vs. 100%), the dose reduction rate was higher (40% vs. 4.2%) and the incidence of FN was higher (80% vs. 22.9%) (Table 4). Although the number of patients was small, it cannot be concluded that TC therapy is applicable for patients aged 65 years or older. Loibl et al. (21) investigated tolerability against taxane-based adjuvant therapy by age, in which the incidences of leukopenia and neutropenia increased with age, but the incidence of FN was similar. Regarding non-hematological toxicity, there was no age-related difference in the incidence of G1–2 fatigue, but the incidence of G3–4 fatigue was significantly higher in the elderly patients. Regarding skin eruption, there was no age-related difference in the incidence of G1–2, but that of G3 or severer skin eruption was significantly higher in the elderly patients (21). Although simple comparison with TC in the above reports is difficult because the regimen was different, these previous reports may support our study results regarding the feasibility of taxane-containing regimens for elderly patients. No significant difference was noted in non-hematological toxicity between the age groups, which may have been due to the small number of patients.

This study confirmed that TC therapy can be safely performed in Japanese. Regarding hematological toxicity, since FN developed at a relatively high rate (28.3%), the use of prophylactic antibiotics should be considered. Regarding non-hematological toxicity, no severe edema developed, but skin eruption accompanied by pruritus appeared in about half of the patients, for which the establishment of supportive therapy may be necessary. On profiling adverse effects by age, the incidence of hematological toxicity markedly increased in patients aged 65 years or older, decreasing the treatment completion rate. The tolerability of patients aged

65 years or older is not favorable, and administration should be carefully decided upon.

Conflict of interest statement

None declared.

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Docetaxel Followed by Fluorouracil/Epirubicin/Cyclophosphamide as Neoadjuvant Chemotherapy for Patients with Primary Breast Cancer

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Objective: This multicenter, open-label, single-arm, Phase II study assessed the efficacy of a neoadjuvant chemotherapy with docetaxel (75 mg/m^2 q3w) followed by 5-fluorouracil 500 mg/m^2, epirubicin 100 mg/m^2 and cyclophosphamide 500 mg/m^2 q3w in patients with early-stage breast cancer.

Methods: Women with resectable breast cancer (T1c–3 N0 M0 or T1–3 N1 M0) were enrolled. Before surgery, patients received four cycles of docetaxel followed by four cycles of 5-fluorouracil, epirubicin, and cyclophosphamide. The primary endpoint was the pathological complete response (pCR) rate defined for the breast alone, assessed by a central review committee. Secondary endpoints included clinical response and safety.

Results: One hundred and thirty-seven patients were enrolled. Of the 132 patients assessable for pathologic response, 23% (95% confidence interval, 16–31%) experienced a pathological complete response and 6% (95% confidence interval, 3–12%) had a near pathological complete response (few remaining cancer cells), resulting in a quasi-pathological complete response of 29% (95% confidence interval, 21–37%). Clinical response rate following the initial docetaxel regimen was 64%. The overall clinical response rate after completion of 5-fluorouracil, epirubicin, and cyclophosphamide was 79%; breast-conserving surgery was performed in 79% of patients. More patients with triple-negative disease (estrogen/progesterone receptors negative; human epidermal growth factor 2 negative) experienced a pathological complete response [14/29, (48%); 95% confidence interval, 29–68%] versus those with other molecular subtypes. The safety profile was acceptable.

Conclusions: Eight cycles of neoadjuvant chemotherapy—docetaxel followed by 5-fluorouracil, epirubicin, and cyclophosphamide—are tolerable and conferred high rates of pathological complete response and breast-conserving surgery. Patients with triple-negative disease were more likely to achieve pathological complete response versus other subtypes, suggesting that selecting appropriate neoadjuvant chemotherapy based on molecular subtype could be possible.

Key words: breast neoplasms – neoadjuvant therapy – FEC protocol – docetaxel

INTRODUCTION

Neoadjuvant chemotherapy has been widely used for patients with operable breast cancer to increase the chance of breast conservation (1–7). Furthermore, response to neoadjuvant treatment can provide important information on long-term survival outcomes. Pathological complete response (pCR) in the breast and axillary lymph nodes predicts a favorable prognosis, whereas a lack of pCR in the breast and node-positive status do not (6,7). This implies the possibility of tailoring subsequent treatment according to the response to initial treatment (7–12). In addition, correlative studies of tumor samples before and after treatment may provide information on markers that could predict response or resistance to treatment (13–16).

Results from the National Surgical Adjuvant Breast and Bowel Project (NSABP) Protocol B-18 trial demonstrated the impact of neoadjuvant chemotherapy in patients with operable early-stage breast cancer (17). The protocol-specified anthracycline-containing regimen—four cycles of doxorubicin and cyclophosphamide (AC)—resulted in an increased likelihood of breast-conserving surgery (BCS) compared with no neoadjuvant chemotherapy. The study established pCR as a prognostic marker for long-term disease-free survival (DFS) and demonstrated that there was no difference in survival if chemotherapy was administered before or after surgery. Subsequent studies, such as the Aberdeen trial, have demonstrated the benefit of the sequential addition of taxanes to neoadjuvant anthracycline regimens (5). The NSABP Protocol B-27 trial demonstrated that, compared with neoadjuvant AC alone, the addition of sequential docetaxel doubled the pCR rate, increased the clinical complete response rate (RR) and increased the proportion of patients with negative axillary nodes (7–18).

We previously conducted a Phase II study to evaluate the clinical and pathological response and safety of the FEC regimen (5-fluorouracil, epirubicin and cyclophosphamide) followed by docetaxel as neoadjuvant chemotherapy in Japanese women with early-stage breast cancer [Japan Breast Cancer Research Group (JBCRG) 01 trial]. The results of this study have been reported previously (19). Although the pCR rate was 16% and BCS was possible for 85% of patients, there were some safety concerns, with 18% of patients experiencing febrile neutropenia and 41% of patients experiencing Grade 1/2 peripheral edema (no Grade 3/4 events observed) following the docetaxel regimen (unpublished data). Disease progression occurred in 6% of patients after the completion of all planned treatment (unpublished data).

In an effort to achieve a higher pathological RR with an improved safety profile, we decided to evaluate the efficacy and safety of docetaxel followed by FEC (JBCRG 03 trial)—the reverse of the sequence of chemotherapy used in the JBCRG 01 trial (19). The clinical and pathological effects and the toxicity profile of this regimen are presented here, and the results of predictive marker analyses are discussed.

PATIENTS AND METHODS

Patient Eligibility

This was a multicenter, open-label, single-arm, Phase II study that recruited patients via central registration. Japanese women aged 20–59 years with histologically proven early-stage breast cancer (T1c–3 N0 M0 or T1–3 N1 M0) were enrolled. No prior chemotherapy, radiotherapy, hormonal therapy or immunotherapy was allowed. Other inclusion criteria were Eastern Cooperative Oncology Group performance status 0–1; white blood cell count 4000–12 000/mm^3; neutrophil count $\geq$ 2000/mm^3; platelet count $\geq$ 100 000/mm^3; hemoglobin $\geq$ 9.5 g/dl; serum bilirubin $\leq$ 1.25 times upper limit of normal (ULN); creatinine $\leq$ 1.5 times ULN and aspartate aminotransferase and alanine aminotransferase $\leq$ 1.5 times ULN. Patients with congestive heart failure or left ventricular ejection fraction $\leq$60% were excluded. Patients were also excluded if they had confirmed infection; serious concomitant illness such as severe cardiovascular disease, uncontrolled diabetes, malignant hypertension or hemorrhagic disease; active concomitant malignancy; brain metastasis; peripheral neuropathy; history of edema with severe drug allergy; or previous long-term corticosteroid therapy. Pregnant or lactating women were excluded. Mammography, ultrasonography, magnetic resonance imaging or computed tomography was used to assess the presence of tumors. Baseline evaluations included complete blood cell and platelet count, routine blood chemistry and liver function tests, chest X-ray, bone scan, electrocardiogram and echocardiogram.

The local ethics committee or institutional review board approved the study at each institution. All patients gave written informed consent to participate. The protocol was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice.

Treatment

Four cycles of docetaxel (75 mg/m^2) administered intravenously (i.v.) every 21 days were followed by four cycles of FEC (5-fluorouracil 500 mg/m^2, epirubicin 100 mg/m^2 and cyclophosphamide 500 mg/m^2) administered i.v. on Day 1 every 21 days before surgery. Premedication was administered based upon each physician's decision to prevent edema, nausea and allergic reactions (e.g. dexamethasone 12 mg i.v. and/or granisetron 4 mg i.v. on Day 1, and oral dexamethasone 8 mg on Days 2 and 3 of docetaxel treatment; dexamethasone 24 mg i.v. on Day 1 and oral dexamethasone 8 mg on Days 2–6 with the FEC regimen). Administration of granulocyte colony-stimulating factor and antibiotics was left to the judgment of each investigator.

Clinical Response Assessment

Tumor assessments were performed within 4 weeks before docetaxel treatment, after completion of docetaxel treatment

and before surgery. Tumor response was assessed using the modified Response Evaluation Criteria in Solid Tumors guidelines (in which confirmatory scans/assessments were not required due to the timing of surgery), for patients who had measurable lesions.

Central Pathologic Assessment

Hematoxylin and eosin-stained slides were prepared from core needle biopsy and surgical specimens from the primary tumor. All surgical specimens were cut in 5 mm interval and all surfaces were microscopically examined in each institution. Pathological response of chemotherapy was assessed by a central review committee consisting of three pathologists who used criteria established by the Japanese Breast Cancer Society. pCR was defined as necrosis and/or disappearance of all tumor cells, and/or the replacement of cancer cells by granulation and/or fibrosis. If only ductal components remained, the pathological response was described as a pCR. Near pCR was defined as extremely high grade marked changes approaching a complete response, with only a few remaining isolated cancer cells (19). Quasi-pCR (QpCR) was the total of both pCR and near pCR. The central review committee evaluated the pathological responses independently from local pathologists. This committee was blinded to the local pathologists' reports. Patients who did not have surgery because of disease progression were considered not to have a pCR.

Hormone Receptor and Human Epidermal Growth Factor 2 Overexpression

Estrogen receptor (ER) and progesterone receptor (PgR) status was determined by immunohistochemistry (IHC) before docetaxel treatment at each participating institute.In general, tumors with more than 10% positively stained tumor cells were classified as positive for ER and PgR. The human epidermal growth factor 2 (HER2) status of the tumor was also determined at each institute by IHC or by fluorescence *in situ* hybridization (FISH) analysis. HER2-positive tumors were defined as those scoring 3+ with IHC staining or testing positive by FISH. HER2-negative tumors were defined as those scoring 0−1+ with IHC or scoring 2+ with IHC and testing negative by FISH.

Surgery and Radiotherapy

Following chemotherapy and clinical assessment of response, patients underwent surgery. If the tumor was too large or invasive for BCS, a modified radical mastectomy was recommended. Careful pathological assessment of tumor margins was performed in accordance with the Japanese Breast Cancer Society criteria (20). Sentinel lymph node biopsy was performed to confirm disease stage or to avoid surgical axillary dissection. Autologous or heterologous reconstructive surgery was performed depending on the patient's requirements and health status. All patients who underwent BCS were given standard radiotherapy to the remaining ipsilateral breast tissue after surgical recovery. For patients diagnosed as sentinel node negative and thus not requiring axillary dissection; radiotherapy to the axilla was allowed.

Toxicity and Dose Modification

Toxicities were evaluated according to the National Cancer Institute Common Terminology Criteria for Adverse Events (version 3) throughout treatment with docetaxel and FEC before surgery. Treatment could be postponed for a maximum of 2 weeks only for severe toxicity. If the adverse event (AE) did not improve during this period, chemotherapy was discontinued and surgery was recommended. Dose reductions were permitted for docetaxel from 75 to 60 mg/m^2 and for epirubicin from 100 to 75 mg/m^2 in cases of febrile neutropenia or Grade 3/4 non-hematologic toxicities, except for nausea, vomiting and fatigue.

Statistical Methods

The primary endpoint was the pCR rate. Before the initiation of the current study, the pCR rate for non-taxane anthracycline regimens ranged from 12.8% (NSABP Protocol B-27) (18) to 15.4% (Aberdeen trial) (5). Previously, we had conducted JBCRG01 trial to evaluate the pCR rate defined for breast disease (19). Therefore, in order to detect improvement in the pCR rate in the same definition of our previous study, a sample of 119 patients was required according to binominal distribution, with a one-sided threshold pCR rate of 12%, an expected pCR rate of 22%, an α error of 5% and a β error of 10%. The target number of patients for recruitment was therefore 119, so assuming that 5% of patients would not be evaluable, we planned to enroll 130 patients. Secondary endpoints included safety, clinical RR, rate of BCS, DFS, overall survival and a subset analysis according to biomarkers. Pathological and clinical RRs were calculated with 95% confidence intervals (95% CIs), with each complete RR based on a binominal distribution. Pathological response was evaluated by hormone receptor status and HER2 status. A multiple logistic regression analysis was performed to examine which factors (menopausal status, tumor size, ER and PgR status, HER2 status and clinical response to docetaxel and FEC) were associated with pCR and QpCR.

RESULTS

Patients Characteristics and Treatment

Enrollment took place from October 2005 through October 2006. One hundred and thirty-seven patients were enrolled. Two patients did not receive study treatment because of early withdrawal of consent; therefore, 135 patients were evaluable for safety and clinical response. These evaluable

Table 1. Patients' characteristics

Characteristic	Value[a]
Number of evaluable[b] patients	135
Age (years)	
Median	46
Range	24–62
Performance status, *n* (%)	
0	133 (99)
1	2 (1)
Menopausal status, *n* (%)	
Premenopausal	94 (70)
Postmenopausal	41 (30)
Clinical tumor stage, *n* (%)	
T1	13 (10)
T2	98 (73)
T3	24 (18)
Clinical nodal stage, *n* (%)	
N0	62 (46)
N1	73 (54)
ER status, *n* (%)	
Positive	86 (64)
Negative	46 (34)
Unknown	3 (2)
PgR status, *n* (%)	
Positive	63 (47)
Negative	70 (52)
Unknown	2 (1)
HER2 status,[c] *n* (%)	
0	21 (16)
1+	63 (47)
2+	20 (15)
3+	31 (23)

ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; PgR, progesterone receptor.
[a]Percentages may not add up to 100% because of rounding.
[b]Number of patients evaluable for safety and clinical response.
[c]Evaluated by immunohistochemistry.

patients included two patients aged 60 and 62 years (included because their age was not considered to influence the evaluation). Two patients were lost to follow-up before surgery, thus 133 patients were evaluable for surgical response. A total of 132 patients were evaluable for pathological response; one patient was excluded owing to lack of confirmation of invasive carcinoma (following the pathologic central review) due to inadequate samples from core needle biopsy before study treatment.

The patient characteristics are summarized in Table 1. Thirty patients (22%) had triple-negative disease, defined as ER-negative, PgR-negative and HER2-negative primary breast cancer, including one patient who was lost to follow-up before surgery.

Overall, 98 patients (73%) completed the planned eight cycles of treatment without dose reductions or study discontinuation. A total of 115 (85%) and 106 (82%) patients completed all four planned treatment cycles of docetaxel and FEC, respectively; dose reductions were necessary in 9 (7%) and 17 (13%) patients, respectively. The majority of the dose reductions were attributable to toxicities, particularly febrile neutropenia during treatment with FEC (10 versus 2 patients during docetaxel treatment). Dose reductions due to neutropenia were required by three patients each during the docetaxel and FEC regimens. Eleven (8%) and six patients (5%), respectively, discontinued treatment during docetaxel and FEC therapy because of toxicities (five patients discontinued during both regimens) or disease progression (six patients during docetaxel and one patient during FEC). The mean dose intensities were 24.2 and 30.3 mg/m^2/week for docetaxel and epirubicin, respectively.

Toxicities

The incidence of treatment-related AEs is summarized in Table 2. Neutropenia was the most common Grade 3/4 treatment-related AE and was observed in 44% and 60% of patients during docetaxel and FEC therapy, respectively. Overall, 67% and 15% of patients experienced at least one episode of Grade 3/4 neutropenia or febrile neutropenia, respectively. For non-hematologic toxicities of any grade, rash, sensory neuropathy, edema, muscle pain and joint pain occurred more frequently during docetaxel treatment than with FEC. Conversely, the frequency of gastrointestinal symptoms, such as nausea, vomiting and anorexia, was higher with FEC than with docetaxel. The frequency of Grade 1/2 peripheral edema was similar during exposure to docetaxel (33%) and FEC (29%); no patient had Grade 3/4 edema. Grade 3/4 non-hematologic toxicities, including gastrointestinal disturbances, were infrequent during both docetaxel and FEC. No fatal AEs were reported.

Clinical Response to Treatment

The overall clinical RR was 79% (106/135; 95% CI, 71–85%), with a clinical complete RR of 21% (29/135), a partial RR of 57% (77/135) and a disease progression rate of 5% (7/135). The clinical RR following the initial docetaxel regimen was 64%. The clinical responses to treatment with docetaxel followed by FEC according to response to initial docetaxel are shown in Table 3. Eight of the 135 patients (6%) progressed during docetaxel administration; 2 of 135 patients (1%) had disease progression during FEC. Of the 30 patients with triple-negative disease, 7 patients were observed to have disease progression following docetaxel treatment. One of the 17 patients with ER-positive, PgR-negative and HER2-negative tumors had disease

Table 2. Treatment-related adverse events

Adverse event, *n* (%)	DOC ($n = 135$)		FEC ($n = 29$)		Overall ($n = 35$)	
	All grades	Grade 3/4	All grades	Grade 3/4	All grades	Grade 3/4
Non-hematologic toxicities						
Infection with neutropenia	6 (4)	2 (1)	3 (2)	2 (2)	9 (7)	4 (3)
Fever	15 (11)	0	13 (10)	1 (1)	22 (16)	1 (1)
Infection (other)	3 (2)	1 (1)	2 (2)	0	4 (3)	1 (1)
Fatigue	82 (61)	0	84 (65)	2 (2)	98 (73)	2 (1)
Nausea	52 (39)	1 (1)	102 (79)	3 (2)	108 (80)	4 (3)
Vomiting	19 (14)	1 (1)	51 (40)	3 (2)	61 (45)	4 (3)
Anorexia	53 (39)	1 (1)	86 (67)	2 (2)	91 (67)	2 (1)
Stomatitis	50 (37)	1 (1)	51 (40)	0	68 (50)	1 (1)
Diarrhea	39 (29)	1 (1)	20 (16)	0	46 (34)	1 (1)
Phlebitis	2 (1)	1 (1)	2 (2)	0	4 (3)	1 (1)
Alanine aminotransferase	36 (27)	0	50 (39)	2 (2)	57 (42)	2 (1)
Aspartate aminotransferase	19 (14)	0	34 (26)	1 (1)	40 (30)	1 (1)
Nail changes	2 (1)	0	33 (26)	1 (1)	33 (24)	1 (1)
Weight loss	5 (4)	0	6 (5)	1 (1)	8 (6)	1 (1)
Creatinine	4 (3)	1 (1)	6 (5)	0	7 (5)	1 (1)
Edema	44 (33)	0	37 (29)	0	55 (41)	0
Hematologic toxicities						
Neutropenia	60 (44)	59 (44)	91 (71)	77 (60)	100 (74)	91 (67)
Leukopenia	69 (51)	50 (37)	101 (78)	66 (51)	108 (80)	76 (56)
Thrombocytopenia	13 (10)	0	28 (22)	2 (2)	31 (23)	1 (1)
Anemia	66 (49)	0	99 (77)	1 (1)	106 (79)	1 (1)
Febrile neutropenia	9 (7)	9 (7)	15 (12)	15 (12)	20 (15)	20 (15)

DOC, docetaxel; FEC, 5-fluorouracil, epirubicin and cyclophosphamide.

Table 3. Clinical response to DOC followed by FEC according to response to initial DOC treatment ($n = 135$)

Clinical response,[a] *n* (%)	Total[b]	Responder	Non-responder
Response to DOC			
Responder	87 (64)	79 (58)	8 (6)
Non-responder	48 (36)	27 (20)	21 (16)

[a]Overall response was confirmed after completion of chemotherapy in comparison with before docetaxel treatment.
[b]Percent value of each column was calculated by dividing by the total number of the evaluable patients ($n = 135$).

progression; while of the 53 patients with ER-positive, PgR-positive, and HER2-negative tumors and of the 9 patients with ER-positive, PgR-positive, and HER2-positive tumors, no patient had disease progression during docetaxel treatment. Among those with triple-negative disease, the majority of patients with disease progression after initial docetaxel were premenopausal [6/7 patients (86%)] and had solid-tubular carcinoma which characterized by solid cluster of cancer cells with expansive growth forming sharp borders [4/7 patients (57%)], as assessed using the Japanese Breast Cancer Society histological classification of breast tumors (21) (Table 4). Excluding the differences outlined above, there were no differences between patient and tumor characteristics for those with progressive disease versus non-progressive disease.

Twenty-seven of 48 non-responders to docetaxel (56%) had a response to FEC treatment; however, 8 of 87 responders to docetaxel (9%) showed no improvement in response with FEC treatment. Following chemotherapy, BCS was performed for 105 of 133 assessable patients (79%).

Pathological Response and Predictive Factors to Treatment

The primary endpoint—pCR rate—was 23% (95% CI, 16–31%). A near pCR rate of 6% (95% CI, 3–12%) resulted

Table 4. Clinical and pathologic characteristics of triple-negative breast cancer[a] for patients with progressive disease versus patients without progressive disease, following initial docetaxel therapy

Characteristic	Without PD	PD
No. of evaluable patients	23	7
Age, years		
Median	43	46
Range	(30–62)	(29–53)
Menopausal status, *n* (%)		
Premenopausal	15 (65)	6 (86)
Postmenopausal	8 (35)	1 (14)
Tumor stage		
T1	2 (9)	0
T2	14 (61)	5 (71)
T3	7 (30)	2 (29)
Nodal stage, *n* (%)		
N0	13 (57)	3 (43)
N1	10 (43)	4 (57)
Tumor type, *n* (%)		
Solid-tubular carcinoma	6 (26)	4 (57)
Papillotubular carcinoma	5 (22)	3 (43)
Scirrhous carcinoma	3 (13)	0
Unspecified invasive carcinoma	9 (39)	0

PD, progressive disease.
[a]Triple-negative tumors were defined as ER-negative, PgR-negative and HER2-negative primary breast cancer.

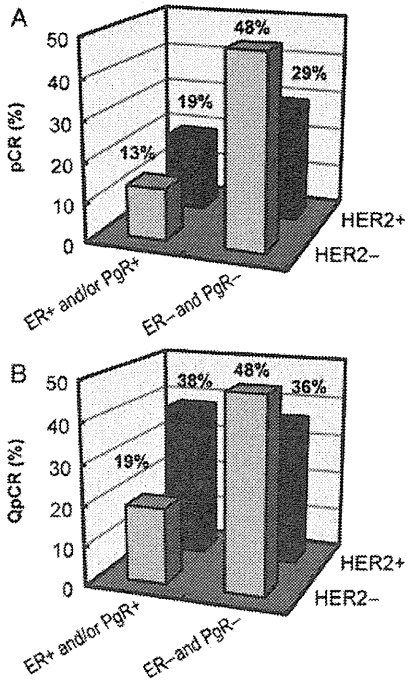

Figure 1. (A) Relationship between pCR versus HER2 and ER/PgR status following DOC and FEC ($n = 129$). (B) Relationship between QpCR versus HER2 and ER/PgR status following DOC and FEC ($n = 129$). Three patients were excluded from evaluable patients for pathologic response ($n = 132$) because of their unknown hormone receptor status. There were no near pCR case observed in triple-negative (ER−, PgR− and HER2−) diseases. DOC, docetaxel; ER, estrogen receptor; FEC, 5-fluorouracil, epirubicin, and cyclophosphamide; HER2, human epidermal growth factor receptor 2; pCR, pathologic complete response; PgR, progesterone receptor; QpCR, quasi-pathologic complete response.

in a QpCR rate of 29% (95% CI, 21–37%) when combined with the pCR. Pathological response of each subset population according to their hormone receptor and HER2 status is summarized in Fig. 1A and B. Patients with triple-negative disease had the highest pCR rate of 48% (95% CI, 29–68%). Near pCR was not observed in triple-negative disease. Patients with HER2-positive, ER-negative and PgR-negative tumors had a pCR rate of 29% (95% CI, 8–58%) and a QpCR rate of 36% (95% CI, 13–65%); patients with HER2-positive and ER-positive and/or PgR-positive tumors had a pCR rate of 19% (95% CI, 4–46%) and a QpCR rate of 38% (95% CI, 15–65%). Patients with HER2-negative and ER-positive and/or PgR-positive tumors had the lowest pCR and QpCR rates (13%; 95% CI, 6–23% and 19%; 95% CI, 10–30%, respectively). One of the seven patients who experienced clinical disease progression with initial docetaxel treatment had a QpCR following FEC.

The relationship between tumor pathological feature and pCR rate is shown in Table 5. The only variable found to be significantly associated with a pCR after docetaxel treatment was ER status.

Survival outcomes will be reported when the 5-year follow-up has been completed for this study.

DISCUSSION

This is the first report to evaluate the effectiveness of an initial docetaxel regimen for neoadjuvant therapy of Japanese patients with early-stage breast cancer. An additional component of the study was to analyze the data according to hormone receptor and HER2 status. Recently, Wildiers et al. (22) reviewed four adjuvant trials which had demonstrated the taxane-first regimens were favorable in terms of the relative drug dose intensity achieved. Also they mentioned larger non-randomized adjuvant studies for a series of 284 patients who first received three cycles of FEC followed by three cycles of docetaxel, the mean relative dose intensity was 91% for FEC and 76% for docetaxel, whereas in another series of 378 patients who received three cycles of docetaxel followed by four cycles of EC (epirubicin plus cyclophosphamide), a median docetaxel dose intensity of 100% was achieved. Therefore, they concluded such data suggest that the administration of a taxane first, followed by an anthracycline, may be preferable in line with the Norton–Simon hypothesis (23). In the JBCRG 01 study, the largest study to date to evaluate neoadjuvant chemotherapy in this patient population, the clinical and pathological responses

Table 5. Predictive variables for pCR before and following chemotherapy

Variables	Before treatment			After DOC			After FEC following DOC		
	OR	95% CI	*P* value	OR	95% CI	*P* value	OR	95% CI	*P* value
Menopausal status: pre (versus post)	1.5	0.94–2.40	0.0923	1.52	0.94–2.47	0.0867	1.42	0.87–2.31	0.1575
Tumor size: ≥3 cm (versus <3 cm)	1.51	0.94–2.41	0.0881	1.45	0.90–2.34	0.1266	1.56	0.96–2.52	0.0724
ER: negative (versus positive)	0.58	0.32–1.03	0.0650	0.51	0.28–0.95	0.0331	0.58	0.32–1.05	0.0709
PgR: negative (versus positive)	0.66	0.34–1.28	0.2211	0.72	0.37–0.95	0.3408	0.65	0.33–1.27	0.2083
HER2: 3+ (versus <3+)	1.32	0.76–2.28	0.3251	1.41	0.80–2.47	0.2360	1.39	0.80–2.41	0.2445
Clinical response to DOC									
Response (versus no response)	—	—	—	0.64	0.38–1.07	0.0875	—	—	—
Clinical response to FEC following DOC									
Response (versus no response)	—	—	—	—	—	—	0.58	0.29–1.14	0.1160

CI, confidence interval; OR, odds ratio; pCR, pathologic complete response.

and safety of FEC followed by docetaxel were investigated (19). The eligibility criteria, treatment dose and distribution of patient characteristics (menopausal status, tumor stage, hormone receptor status and HER2 status) studied in the JBCRG 01 trial were similar to those investigated in the present JBCRG 03 study (19). The incidences of Grade 3/4 neutropenia and febrile neutropenia observed in the current study were similar to those reported in the JBCRG 01 trial (19). However, the rate of Grade 1/2 edema during docetaxel treatment was lower in the present study (33%) than in the JBCRG 01 study (41%), suggesting that docetaxel might be better tolerated when given up front than when administered after completion of prior chemotherapy. Further studies are warranted to assess quality of life and the incidence of edema in order to confirm the effect of administering docetaxel as the initial therapy.

Many different neoadjuvant chemotherapy schedules and dose regimens are used in clinical practice. The NSABP Protocol B-18 trial, which compared AC treatment before and after surgery, reported no difference in DFS between the two approaches (17). However, the rate of BCS was greater with neoadjuvant AC chemotherapy, and the prognosis of patients who obtained a pCR was also better with this treatment regimen (17). Several other regimens have been evaluated in an effort to increase the pCR rate. The addition of a taxane to an anthracycline-containing regimen has been shown to improve the pCR and clinical RRs (5,18). Furthermore, excellent results have been reported by the MD Anderson Cancer Center using a regimen of paclitaxel plus trastuzumab followed by FEC plus trastuzumab in patients with operable breast cancer and HER2 overexpression (24). However, few studies have evaluated initial taxane therapy followed by an anthracycline-containing regimen in this indication (24). Thus, it was decided to evaluate such a reverse regimen and to analyze the findings according to molecular subtypes. Importantly, the primary endpoint—pCR rate—achieved in the present study was 23% (95% CI, 16–31%), far exceeding our estimate of 12% (19). Even though the pCR rate here cannot be directly compared with the results from the JBCRG 01 trial (pCR rate: 12%, QpCR rate: 25%), the pCR rate from this study is a favorable result considering the similar patient characteristics in both trials (19).

The overall clinical RR of 79% was similar to that reported in the JBCRG 01 trial (74%) (19). Furthermore, the clinical RR following the initial docetaxel regimen was 64%, similar to the clinical response following the initial FEC regimen in the JBCRG 01 trial (61%) (19). The clinical RR following the initial docetaxel regimen, however, is lower in this study than those reported in other studies (71.7–85%) (25,26). It could be hypothesized that the clinical response might be influenced by the lower dose of docetaxel used in this study (75 mg/m^2) compared with the 100 mg/m^2 dose used in previous studies (25,26).

The rate of BCS observed in our study (79%) was similar to that reported in the JBCRG 01 trial (85%) (19). Unfortunately, the overall disease progression rate (5%) was not lowered by the use of docetaxel followed by FEC in this study, and was similar to that seen in the JBCRG 01 trial (6%) (19).

Although 7 of the 29 patients with triple-negative disease had disease progression during the initial docetaxel regimen, 14 of the 22 patients without disease progression (64%) achieved a QpCR. This QpCR rate is markedly higher compared with previous findings (27).

Our results indicate that if patients with triple-negative disease who experienced disease progression following initial docetaxel therapy were excluded, the pCR rate for this group of patients would have been higher. We thus compared the clinical and pathological characteristics between patients with triple-negative disease who experienced disease progression following the initial docetaxel regimen with those who did not have disease progression. However, no

significant differences in patient or tumor characteristics were seen between these patient groups. It was noted, however, that six of seven premenopausal patients (86%) and four of seven patients (57%) with solid-tubular carcinoma had disease progression following docetaxel therapy. Given the high incidence of disease progression among patients with triple-negative disease who had solid-tubular subtype tumors, this phenotype could be used in future studies to predict which patients are more likely to experience progressive disease following docetaxel therapy. Accordingly, the identification of patients with hormone receptor-positive and HER2-negative disease would also enable the selection of patients who are more likely to benefit from neoadjuvant chemotherapy. Thus, studying patients' molecular subtypes, and selecting appropriate chemotherapy regimens accordingly, has the potential to provide superior results to those of the JBCRG 03 trial.

Recently, it has been shown that basal-like breast cancer defined by five biomarkers [epidermal growth factor receptor (EGFR), cytokeratin 5/6 (CK5/6), ER, PgR and HER2 status] provides a more specific definition of basal-like breast cancer that predicts survival better than the triple-negative phenotype (27,28). In patients treated with anthracycline-based chemotherapy, tumors found to be positive for the basal markers corresponded to a cohort of patients with a significantly worse outcome (29). Thus in future trials, it may be beneficial to assess EGFR and CK5/6 status in patients with triple-negative disease to help predict patient survival.

Interestingly, the pCR rate (27%) following neoadjuvant chemotherapy in patients with HER2-negative breast cancer was higher in this study than in the JBCRG 01 study (14%), suggesting that this subpopulation may benefit from initial docetaxel treatment. Conversely, a lower QpCR rate was observed in HER2-positive patients (37%) in this study than in the JBCRG 01 trial (52.8%). This suggests that initial anthracyclines may be required for HER2-positive disease. A study by Buzdar et al. (24) reported that a high pCR rate of 60% was observed in patients with HER2-positive disease treated with the combination of paclitaxel plus trastuzumab followed by FEC plus trastuzumab, indicating that the HER2-positive population in the current study may have benefited further from concomitant trastuzumab therapy. These findings demonstrate the benefit of selecting the most effective chemotherapy regimen according to each patient's molecular subtype and initial response to neoadjuvant treatment.

One limitation of the study was that HER2-positive patients were not treated with trastuzumab, which has been shown to improve outcomes in patients with HER2-overexpressing breast cancer (24). Further studies investigating optimal treatment regimens for different molecular subtypes should include concurrent trastuzumab for patients with the HER2-positive phenotype.

In conclusion, docetaxel followed by FEC as neoadjuvant chemotherapy is a tolerable and effective regimen for patients with early-stage breast cancer. In addition, a high pCR rate made this regimen particularly promising in patients with triple-negative breast cancer. In the future, selection of a neoadjuvant chemotherapy regimen for operable breast cancer may be possible based on molecular subtype.

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Conflict of interest statement

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Pilot study of radiofrequency ablation therapy without surgical excision for T1 breast cancer: evaluation with MRI and vacuum-assisted core needle biopsy and safety management

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Abstract
Background There is increasing demand for minimally invasive treatments for small breast cancer mainly because of the desire for better cosmetic results. Although radiofrequency ablation (RFA) is an attractive approach as a local control method for small breast cancer, the problems of histological effectiveness and safety management remain.
Methods A total of 29 patients including one patient with bilateral breast cancer were enrolled in this study. The mean tumor size of 30 breasts was 12.8 mm (range 5–19 mm). Under general anesthesia, RFA was performed with a Cool-tip RF system (Valleylab, Boulder, CO, USA) after sentinel lymph node biopsy. Postoperative evaluation with magnetic resonance imaging (MRI) and vacuum-assisted core needle biopsy was done 3–4 weeks after RFA before radiotherapy. Ablated tumors were evaluated with hematoxylin–eosin (H&E) and nicotinamide adenine dinucleotide (NADH)-diaphorase staining. If needed, adjuvant chemo and/or endocrine therapy was performed.
Results All patients except one completed one session of RFA. The mean temperature near the center of the tumors was 89.6°C (range 78–100°C). Postoperative MRI showed the ablated zone clearly in all patients. MRI revealed no hypervascularity of the tumors in the ablated zone. Evaluation with H&E staining of the tumors showed remarkable degenerative changes in only three patients. NADH-diaphorase staining showed no viable tumor tissue in 24 patients out of 26 examined. Three patients received small diameter grade 3 skin burns, two on the outside of the thigh from the grounding pad and one on the breast skin. One patient had a breast lesion like a chronic granulomatous mastitis resulting from overreaction of the ablated zone.
Conclusions RFA therapy appeared relevant and applicable for patients with small breast cancer. Because small skin burns were observed as adverse events, close attention should be paid in the course of the RFA procedure.

Keywords Early breast cancer · Radiofrequency ablation · Single-needle electrode · Vacuum-assisted core needle biopsy · NADH-diaphorase staining

Abbreviations

RFA	Radiofrequency ablation
MRI	Magnetic resonance imaging
MDCT	Multi-detector computed tomography
H&E	Hematoxylin–eosin
NADH	Nicotinamide adenine dinucleotide

Introduction

For more than a decade, breast-conserving therapy has been a reliable standard treatment for patients with early breast cancer. Sentinel lymph node biopsy has gained widespread acceptance in recent years as a theoretical method of evaluating axillary lymph node status to avoid unnecessary axillary lymph node dissection in patients with

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clinically node-negative breast cancer. In addition, progress in diagnostic modalities such as mammography, ultrasonography, and magnetic resonance imaging (MRI) mammography has recently enabled increasing detection of small breast tumors with a diameter <1 cm. Thus, surgical treatment of breast cancer has become less invasive, but cosmetic problems remain. There is increasing demand for minimally invasive alternative approaches for patients with small breast cancer.

At present, radiofrequency ablation (RFA) is one of the most promising minimally invasive ablation procedures. RFA was first investigated as a treatment for breast cancer 10 years ago [1]. Thereafter many feasibility studies of RFA followed by surgical resection suggested that RFA would be most effective in tumor death [2–9]. In recent years the authors of several pilot studies have reported the effectiveness of RFA without surgical excision for small breast cancer [10–14]. The purpose of this study was to examine the effect on tumor death and the safety of the RFA procedure using MRI and vacuum-assisted core needle biopsy for patients with T1 breast cancer.

Patients and methods

This study was approved by our institutional review board. All patients received information about this study and signed a written consent form for study participation. The eligibility criteria were as follows: invasive ductal carcinoma established by core-needle biopsy, tumor diameter of 2 cm or less measured by ultrasound, no diffuse microcalcification revealed in mammography, no evidence of extensive ductal spread of cancer or multiple tumors on MRI mammography, and no swelling of axillary lymph nodes proven by ultrasound and contrast-enhanced multidetector computed tomography (MDCT) with 1.3-mm thin slice. Accurate biological tumor characteristics such as estrogen and progesterone receptors and Her-2/neu expression were estimated from tumor tissue taken with core needle biopsy.

Under general anesthesia, a sentinel lymph node biopsy was initially done with a combination method of technetium-99m-labeled phytate (Daiichi Radioisotope Laboratories, Tokyo, Japan) and indigocarmine (Daiichi Pharmaceutical, Tokyo, Japan). In the case of sentinel lymph node metastasis with pathological diagnosis in frozen section, axillary lymph node dissection was performed. A single-needle 17-gauge electrode 10 cm in length with a 3-cm tip exposure (Cool-tip, Valleylab, Boulder, CO, USA) was used for RFA. The sticking point of the breast skin was pricked with the point of a surgical knife 7–8 cm from the tumor. The needle was percutaneously inserted into the tumor diagonally along the direction of the long axis of the tumor under ultrasound guidance. Needle penetration at the center of the tumor was confirmed by two-dimensional ultrasound, and the tip of the needle was placed outside the tumor approximately 5 mm from the edge of the tumor (Fig. 1). About 20–40 ml of 5% glucose solution was injected into the retromammary space and subcutaneous tissue around the tumor to avoid burns of the major pectoral muscle and skin from RFA-induced heat. Thermal ablation was performed using a generator with two grounding pads on each thigh. RFA was started at 5 W output. Output was raised to 10 W 1 min later and

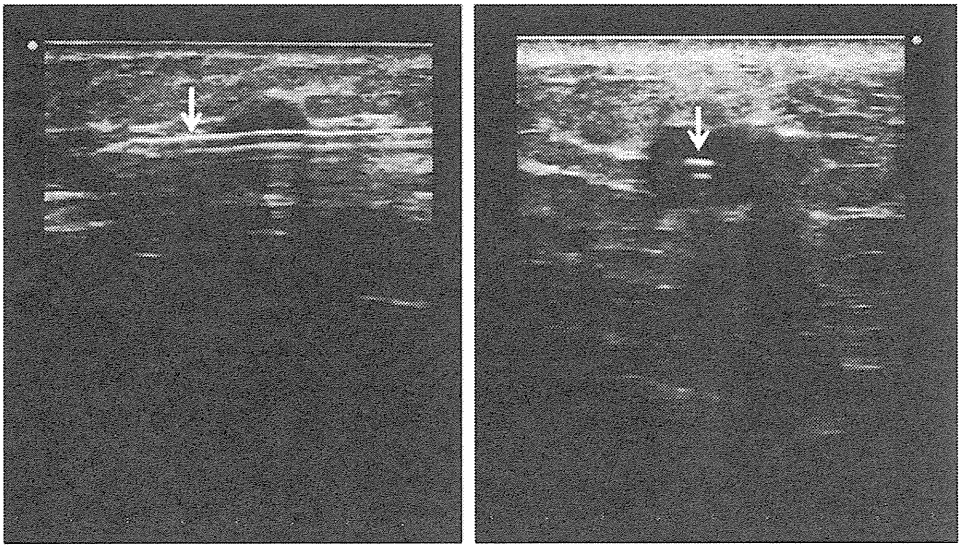

Fig. 1 The needle (*arrows*) penetrating at the center of the tumor as confirmed by two-dimensional ultrasound. *Left* The tip of the needle was placed outside of the tumor approximately 5 mm from the edge of the tumor

thereafter increased by 10 W every 1 min until the generator automatically stopped delivering radiofrequency energy, i.e., took a "break." The skin just above the tumor was cooled with an ice pack from 2 min after the start of RFA until the following morning. The temperature of the tumor was measured with a thermosensor at the tip of the RF needle after the procedure.

Postoperative MRI assessment and histological examination were performed 3–4 weeks after RFA and before radiotherapy. Burak et al. [8] used MRI to study the tumor after RFA and found a good correlation between contrast enhancement and residual tumor. Therefore, MRI can be used to predict complete tumor ablation after RFA [14]. In addition, MRI is valuable for clinical follow-up of breast cancer patients undergoing RFA [15]. Ablated tumor tissues were obtained using a Handy Mammotome (Tyco Health Care, King of Prussia, PA, USA) with an 8G needle under ultrasonography guidance. Vacuum-assisted biopsy with Handy Mammotome can collect tumor tissue easily and abundantly rather than undertaking tumor excision. To evaluate the effects of RFA histologically, nicotinamide adenine dinucleotide (NADH)-diaphorase staining is essential in addition to routine hematoxylin–eosin (H&E) staining and is usually used to judge tissue viability after RFA therapy [1, 2, 5, 6, 9, 13, 16, 17]. With NADH-diaphorase staining, oxidation reaction in the cytoplasm of viable tissue results in a dark blue stain, whereas the nonviable tissue appears pale gray.

After postoperative MRI assessment and histological examination, in the case of an incomplete RFA therapy, the patient was scheduled to undergo salvage partial or total mastectomy. If successful RFA therapy was confirmed, patients received adjuvant radiotherapy (50 Gy ± boost 10 Gy) and if needed, adjuvant systemic therapy.

Results

Twenty-nine patients were enrolled in this study between February 2006 and May 2009. They ranged in age from 38 to 78 years (average 55.9). Axillary lymph node dissection was done in seven patients due to metastasis of sentinel lymph nodes. RFA was done for 30 breast tumors including one patient with bilateral breast tumors that both met the eligibility criteria. Ablation results of the 30 breasts are shown in Table 1. The mean tumor size was 12.8 mm (range 5–19 mm). The mean of the initial impedances was 153.8 Ω (range 99–260 Ω). The mean output was 77.6 W (range 40–125 W). The mean time for RFA was 11.4 min (range 6–20 min). The mean temperature near the center of the tumor was 89.6°C (range 78–100°C). The mean temperature about 1.0–1.5 cm from the edge of the tumors, which was considered the margin of the ablated field, was 70.3°C (range 48–85°C). The patient with the second-highest initial impedance (232 Ω) completed two sessions of RFA. The remaining patients completed one session of RFA.

Table 1 Results of RFA

	No. of breasts
Tumor size on ultrasound (mm)	
10 or less	13
11–15	8
16–20	9
Maximum power (watts)	
50 or less	8
51–100	15
101 or more	7
Ablation time (min)[a]	
10 or less	15
11–15	12
16 or more	2
Temperature of ablated tumor (°C)	
71–80	2
81–90	15
91–100	13
Temperature of ablated margin (°C)	
60 or less	5
61–80	22
81–100	3

[a] Excluding one case due to incomplete ablation during one session

Postoperative MRI clearly showed an ablated zone with surrounding rim enhancement whose shape was close to spherical or elliptical in all patients. The shape of the ablated zone at maximum coronal section of MRI was almost round or oval. The minimum and maximum sizes of the ablated zone in the maximum coronal section were 23 × 22 mm and 60 × 47 mm in diameter, respectively. The mean size of the ablated zone was 38 × 33 mm in diameter. MRI revealed no hypervascularity of the tumor in the ablated zone in any of the patients (Fig. 2).

The mean number of tissue samples obtained from the ablated zone was 9 (range 4–13). The mean number of samples that included tissue from the tumor was 5 (range 0–10). In one patient, none of the tissue samples included tumor tissue. Evaluation with H&E staining of tumors in 29 breasts showed remarkable degenerative changes in three patients. The remaining tumors revealed no remarkable changes and were diagnosed as viable tumor tissue. NADH-diaphorase staining was performed for 26 breast tumors; the first consecutive three patients and the patient in whom no tumor tissue was found during Mammotome biopsy were not included. NADH-diaphorase staining showed no viable tumor tissue in 24 patients. In one patient, viable tumor cells were observed in one sample out

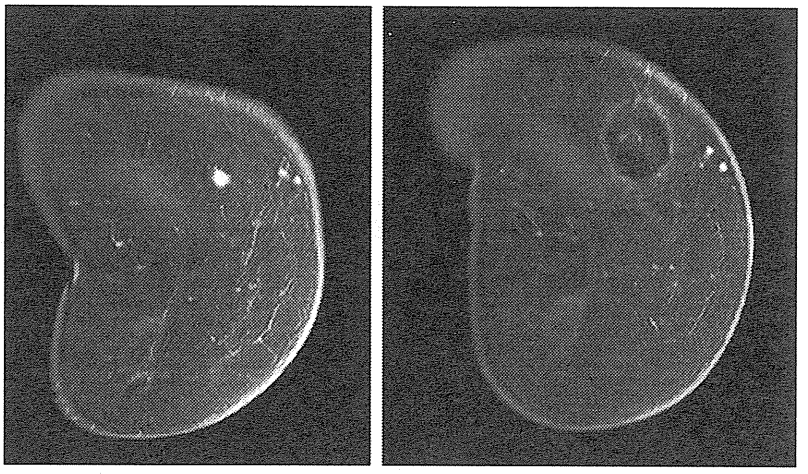

Fig. 2 MRI before (*left*) and after (*right*) RFA. MRI after RFA clearly showed the ablated zone and revealed no hypervascularity of the tumor

of six, and in another patient, viable cells were found in one sample out of ten. Two patients with a few viable tumor cells refused salvage surgical treatment. These patients received adjuvant radiotherapy to the whole breast of 50 Gy plus a 10-Gy boost to the ablated lesion.

Adverse events over grade 2 were observed in four patients. Two patients received small grade 3 burns on the outside of thigh from the grounding pad. One patient received a grade 3 burn of minimal diameter on the breast skin at the top of the ablated zone (Fig. 3), and one patient suffered an overreaction of the ablated zone that was like a chronic granulomatous mastitis (Fig. 4). Her breast lesion was detected 1 year after RFA. She took prednisolone for 1 month, but there was no sign of improvement of the breast lesion. She underwent partial mastectomy of the ablated lesion. Pathological findings of the lesion were fat necrosis with surrounding fibrosis and granulomatous reactions. There was no evidence of cancer cells in the lesion.

Cosmetic results were almost all excellent (Fig. 5) with the exception of one patient with overreaction of the ablated zone. Although median follow-up of 17 months (range 2–41 months) was short, all of the patients were alive without recurrence at the end of follow-up.

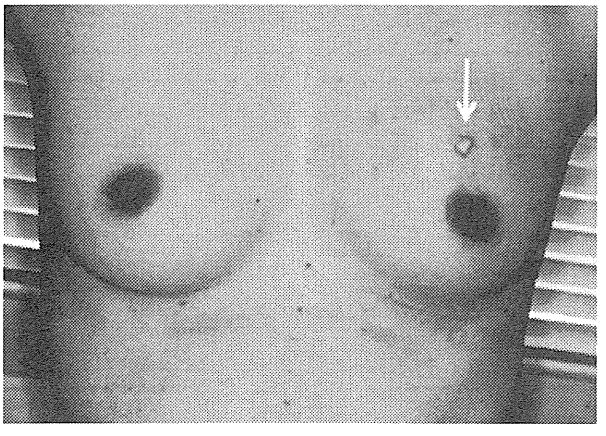

Fig. 3 A minimal diameter grade 3 breast skin burn (*arrow*)

Discussion

Several studies [2, 5, 6, 9, 10, 13, 16, 17] have been performed to investigate the effectiveness of RFA therapy in cancer cell destruction for small breast cancer. In these studies, multiple-needle electrodes such as the LeVeen Needle electrode (Radio Therapeutics, Mountain View, CA, USA) [2, 6] or the Starburst Needle electrode (RITA Medical System, Manchester, GA, USA) [5, 9, 13, 16] were used. On the other hand, Cool-tip single-needle electrode was adopted in some studies [10, 14, 17] including the present study. Thus RFA treatment for breast cancer has been investigated with different types of needle electrodes.

The first feasibility study of RFA in human breast cancer was reported in 1999 [1]. This study was performed in five patients with locally advanced breast cancer immediately followed by mastectomy, and successful results were obtained. Subsequently, several investigators reported feasibility studies of RFA for small breast cancer. In some studies [2–6] the tumor was immediately removed after RFA. Histological evaluations with H&E staining and NADH-diaphorase staining were obtained. The rate of complete coagulative necrosis ranged from 92 to 100% in this series. In other studies [7–9], the tumor was removed 1–4 weeks after RFA. Although tumor viability was assessed on histological examination with only H&E staining, the rate of complete coagulative necrosis ranged from 86 to 100%. Thus several feasibility studies of RFA in human breast cancer showed good results regarding tumor destruction. Therefore nonsurgical pilot studies of RFA therapy for breast cancer have been conducted.

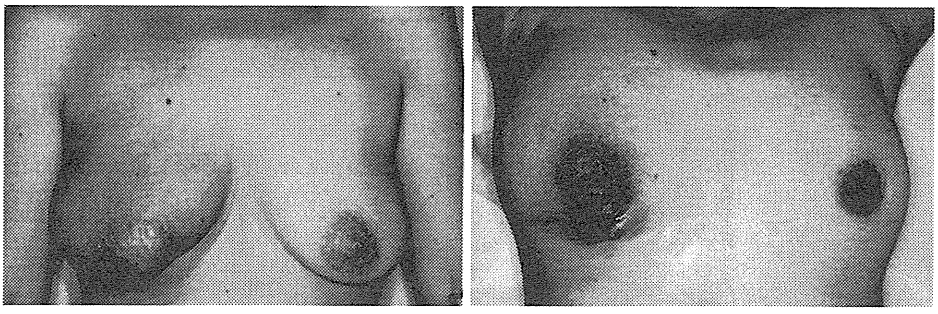

Fig. 4 Overreaction of ablated zone of the breast that was like a chronic granulomatous mastitis

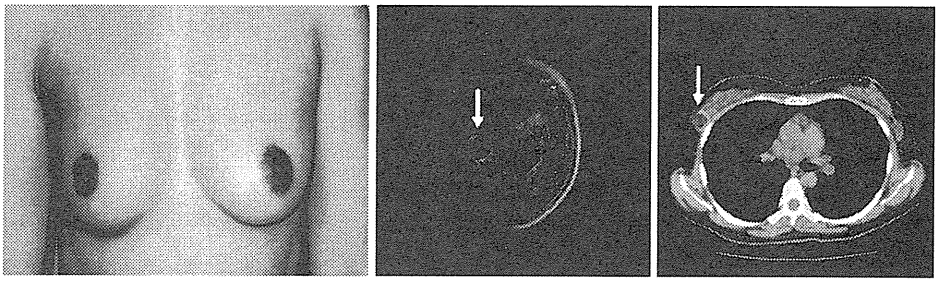

Fig. 5 An excellent cosmetic result (*left*), MRI (*middle*), and MDCT (*right*) of the right breast 30 months after RFA. The ablated lesion was still visible on MRI and MDCT (*arrows*)

The first pilot study to investigate the effectiveness of RFA before radiotherapy without surgical excision for treatment of breast cancer was attempted with three elderly inoperable patients in Italy [10]. A similar study was performed on four elderly inoperable breast cancer patients treated with or without radiotherapy in France [11]. These studies supported the feasibility of RFA treatment for early breast cancer. Thereafter, several pilot studies of RFA therapy for small breast cancer were performed in Japan [12–14]. Earashi et al. [13] and Oura et al. [14] performed RFA therapy followed by breast radiation without surgery in 7 and 52 patients, respectively. They reported successful results of RFA therapy with Mammotome biopsy and cytological assessment, respectively. Based on previous studies using simple aspiration technique with single-needle electrode and following the regular RFA procedure, tumor destruction rates of over 90% can be expected. The aspiration technique under ultrasound guidance with core-needle biopsy and fine-needle aspiration cytology is common for surgeons in our institute. In addition, unlike the sentinel lymph node biopsy, the regular RFA procedure has a very small learning curve. Therefore we planned a nonsurgical pilot study of RFA therapy for early breast cancer patients from the first.

In the present study, we performed the consecutive prospective MRI assessment and Mammotome biopsies without tumor excision after RFA treatment before radiotherapy and chemotherapy for small breast cancer. Single-needle electrode of 10 cm length with 3 cm exposure (Cool-tip) was available. The ablation zone with this sort of needle is normally elliptical and approximately $4 \times 3 \times 3$ cm in diameter. However, the size of the ablation zone measured with MRI was uncertain in the present study. Manenti et al. [17] reported that no significant differences in mean lesion ablation volumes were observed in relation to breast glandular pattern classified as dense, adipose, and mixed patterns. The problem regarding uncertainty of the size of the ablation zone should be investigated in the future. The ablated margin must be at least 1 cm from the tumor in the case of tumors of the maximum size of 2 cm. No histological examination of the ablated margin could be performed in the present study. However, MRI could assure the ablated margin of the tumor to some degree. MRI after the procedure confirmed that the tumor was at the center of the ablated zone in most of our patients. Burak et al. [8] investigated tumor viability after RFA with MRI and found a good correlation between contrast enhancement and residual tumor. MRI can be used to predict complete tumor ablation after RFA treatment for breast cancer in most cases. Fornage et al. [16] suggested that, alternatively, multiple core-needle biopsy specimens taken through and at the periphery of an ablated lesion would confirm the success of RFA. Although the problem of intraductal components in the ablated margin that can only be determined microscopically remains, it is difficult to resolve this issue unless all of the ablated lesion can be removed.

Histological examination with H&E staining was available to assess tumor viability in this study. Evaluation

with H&E staining of tumors in 29 breasts showed remarkable degenerative changes in three patients. There is no established criterion for assessing tumor viability with H&E staining after thermal ablation therapy in breast cancer. Pathologists at each institution would have differing opinions about this issue, which might be one of the reasons for differences in the rate of complete coagulative necrosis with H&E staining between other studies [5, 6, 13, 17] and the present one. On the other hand, NADH-diaphorase staining showed no viable tumor tissue in 24 out of 26 patients in the present study. The complete coagulative necrosis rate of 92% (24/26) was equivalent to that reported in other studies [2, 6, 9, 13, 16, 17] for small breast cancer evaluated with NADH-diaphorase staining. Although tumor tissue samples obtained from Mammotome biopsy do not include the whole tumor, vacuum-assisted biopsy such as a Mammotome biopsy could replace tumor excision to assess the histological effectiveness of RFA therapy in further clinical studies. Two patients with a few viable tumor cells refused salvage surgical treatment. These were violations of the protocol. Because the patients eagerly hoped for nonsurgical therapy, they received adjuvant radiotherapy to the whole breast of 50 Gy plus a 10-Gy boost to the ablated lesion instead of salvage surgery.

Adverse events occurred in 4 out of 29 patients in the present study. Two patients had small areas of grade 3 burns on the outside of the thigh from the grounding pad. Each point of the burns was adjacent to the connection between the cord and the grounding pad in contact with the thigh skin. These burns occurred because the adhesion of grounding pads to the skin became loose at these points due to excessive tension of the cords. The use and proper placement of the grounding pads is a key element in the safe and effective use of the generator, particularly in the prevention of pad-site burns (Fig. 6). On the other hand, a minimal diameter grade 3 burn of breast skin was observed at the top of the ablated zone in 1 out of 30 breasts. In this case, the burn was not detected within 1 week but rather 10 days after RFA. The complication rate of breast skin burn appears to be about 5% (8/172), if one adds the cases described in reports about RFA therapy followed by surgical resection [6]. In studies of RFA without surgical excision [10, 13, 14], the rate was reported at a lower range of 0–2%. More attention during RFA procedure should be paid to avoid skin burns in the case of small breasts with a thin layer of subcutaneous fat tissue. Although an adequate glucose solution was injected into the subcutaneous tissue over the tumor to avoid the skin burn of the breast from RFA-induced heat before the ablation, additional injection may be needed during the ablation if the coagulation time is long, such as over 15 min, because of absorption of the injected solution into the surrounding tissue with the passage of time.

The most severe adverse event occurred in one breast with overreaction of the ablated zone that was like a chronic granulomatous mastitis. Such an adverse event has not previously been reported after RFA treatment for breast cancer. Granulomatous mastitis is a rare disease that predominantly occurs in premenopausal women shortly after their last childbirth [18]. Idiopathic granulomatous mastitis is thought to be an autoimmune reaction to extravasated fat and protein-rich luminal fluid [19]. In this case, an erythema of the breast skin was observed during the early stage of the breast irradiation after RFA, and subsequently the lesion of the breast was observed. It is extremely difficult to predict this type of adverse event, as it is very rare; however, Head and Elliott [20] reported that depo-medrol was instilled into the ablation site to inhibit the fat necrosis resulting from the RFA. This method may become the prophylactic against the adverse event caused by autoimmune reaction.

RFA therapy appeared relevant and applicable for patients with small breast cancer in this study. Next, RFA

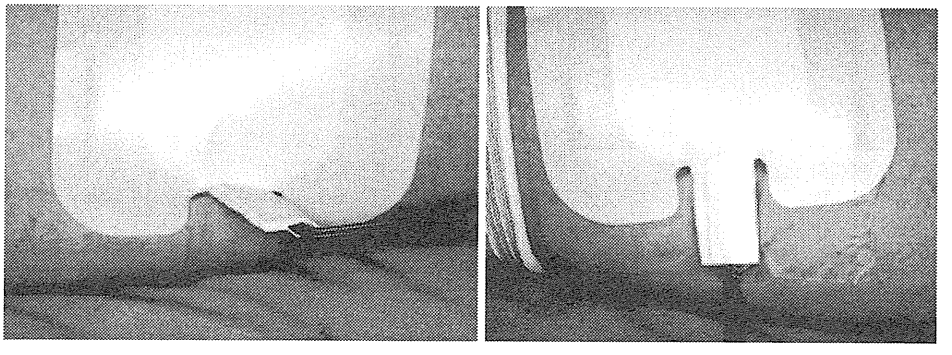

Fig. 6 *Left* Incomplete adhesion of a grounding pad to the skin due to excessive tension of the cord. *Right* Pad-site burns can be prevented by using adhesive tape where the cord connects to the grounding pad