Table 4 Pharmacokinetic parameters for toremifene (dose: toremifene 120 mg) with PXL

Patient no.	$T_{1/2}$ Z (h)	C_{max} (μg/mL)	T_{max} (h)	AUC 12 h (μg h/mL)	AUC inf. (μg h/mL)	V_z/F (L)	CL/F (L/h)	MRT (h)
1	7.3605	2.64	2	24.9775	36.1135	35.2852	3.3229	10.1446
2	42.6484	2.05	2	21.2075	117.2959	62.947	1.0231	61.2399
3	468.634	1.52	2	17.045	895.0701	90.6426	0.1341	675.0959
4	–	1.92	2	18.8975	–	–	–	–
5	41.5018	0.7	7	8.2875	43.859	163.819	2.736	59.349
6	3.9296	1.5	7	15.4525	17.3186	39.2816	6.929	5.9189
7	5.8377	2.91	2	17.3025	22.6478	44.6241	5.2985	7.8881
8	11.2065	1.27	2	14.1025	25.517	76.0322	4.7028	15.2716
9	22.5817	1.75	2	13.1875	41.586	94.0081	2.8856	32.0872
10	7.1388	3.23	2	18.225	26.0258	47.4874	4.6108	9.6959
11	9.2767	2.36	2	16.26	27.9838	57.3911	4.2882	13.5226
12	31.4822	1.95	2	15.36	63.7578	85.4846	1.8821	44.8654
13	–	1.29	3.5	15.555	–	–	–	–
14	–	0.87	3.5	9.7925	–	–	–	–
15	26.0695	1.74	2	18.3675	63.5659	71.0009	1.8878	36.7969
Mean	56.5	1.85	2.9	16.3	115.1	72.3	3.31	81
SD	130.5	0.71	1.8	4.1	247.2	35.1	1.94	188.2
Median	16.9	1.75	2	16.3	38.8	67	3.1	23.7
Maximum	468.6	3.23	7	25	895.1	163.8	6.93	675.1
Minimum	3.9	0.7	2	8.3	17.3	35.3	0.13	5.9

Blood samples were collected on day 32 from the patients given toremifene (120 mg/day) after meal

$T_{1/2}$ *Z* half-life in the terminal phase, C_{max} maximum concentration, T_{max} time to reach maximum concentration, *AUC 12* area under the concentration–time curve up to 12 h after administration, *AUC inf.* area under the concentration–time curve up to infinity time, V_z/F volume of distribution based on the terminal phase, where *F* is the bioavailability, *CL/F* oral clearance, where *F* is the bioavailability, *MRT* mean residence time, *CL tot* total body clearance, V_{ss} volume of distribution at steady state, – not calculated because the terminal phase could not be observed

Eleven patients had positive estrogen receptors. All patients had evaluable lesions, and there was one partial response, eight cases of stable disease, and six cases of progressive disease.

Pharmacokinetic study

We collected blood samples from 15 patients. Pharmacokinetic parameters obtained from 15 patients were analyzed using data obtained on day 1 (Table 2). On day 18, we collected blood samples at two different time points to determine the area under the AUC and C_{max}. The mean AUC of PXL on day 18 was lower than the AUC on day 1 (Fig. 2). No significant intra-patient variability in PXL was observed (data not shown). The mean AUC of PXL without TOR was 8.74 μg h/mL; the mean AUC of PXL with TOR was 7.99 μg h/mL (Table 3). No statistically significant differences in the C_{max} and AUC of PXL were observed ($P > 0.05$). Other pharmacokinetic parameters, such as half-life in the terminal phase, area under the concentration–time curve up to infinite time, total body clearance, and mean residence time were similar in the presence or absence of TOR (Table 4). In the presence of TOR, the AUC of PXL increased in three patients (patient nos. 2, 5, and 15) but decreased in four patients (patient nos. 1, 8, 9, and 11). Inter-patient variability was observed for each PK parameter of PXL. The PK parameters of TOR are presented in Table 4. On day 32, blood samples were obtained from patients who were administered 120 mg TOR daily for 14 days. C_{max} and the AUC of TOR were 1.85 ± 0.71 μg/mL and 115.1 ± 247.2 μg h/mL, respectively. The pharmacokinetic parameters of TOR-1 were similar in the presence and absence of PXL (data not shown).

Feasibility and toxic profile of PXL and toremifene

Adverse events were evaluated in the 15 patients with NCI-CTC ver.2. No specific adverse events greater than grade 3 were observed (Table 5). TOR did not enhance the adverse events of PXL significantly. Neither hematologic nor nonhematologic toxicities of PXL were enhanced in the presence of TOR.

Table 5 Adverse events occurring more than three times at any grade are listed

	Baseline value				PXL				PXL + TOR			
	1	2	3	4	1	2	3	4	1	2	3	4
Nonhematologic												
Nausea–vomiting	1	0	0	0	5	0	0	0	6	1	0	0
Stomatitis	0	0	0	0	2	0	0	0	3	1	0	0
Hair loss	6	4	0	0	8	6	0	0	2	13	0	0
Neuropathy	10	0	0	0	11	0	0	0	11	1	0	0
Myalgia arthralgia	2	0	0	0	3	0	0	0	5	1	0	0
Hot flash	0	0	0	0	14	0	0	0	13	0	0	0
Muscle weakness	3	0	0	0	8	0	0	0	7	2	0	0
Taste disturbance	1	0	0	0	3	0	0	0	3	0	0	0
Edema	0	3	0	0	3	3	0	0	2	3	0	0
Fatigue	0	0	0	0	3	0	0	0	3	0	0	0
Vaginal bleeding	0	0	0	0	0	0	0	0	3	0	0	0
Cough	4	0	0	0	4	0	0	0	4	0	0	0
Hematologic												
Leucopenia	0	0	0	0	4	5	2	0	3	2	1	0
Hemoglobin	3	1	0	0	7	2	0	0	4	4	0	0
Febrile neutropenia	0	0	0	0	0	0	0	0	0	0	1	0
AST	3	1	0	0	6	0	0	0	2	1	0	0
ALT	3	0	0	0	7	0	0	0	3	1	0	0
Bilirubin	1	0	0	0	0	0	0	0	0	0	0	0
r-GTP	3	0	1	0	4	2	0	0	0	5	0	0
ALP	6	1	0	0	9	0	0	0	4	1	0	0
Albumin	2	0	0	0	4	0	0	0	6	0	0	0
Hypercholesterolemia	7	0	0	0	7	0	0	0	4	0	0	0
Hypertriglycemia	5	0	0	0	6	0	0	0	7	0	0	0
Proteinuria	2	0	0	0	5	0	0	0	3	0	0	0
Hematuria	3	0	0	0	4	0	0	0	2	0	0	0

All adverse events were evaluated by NCI-CTC Ver. 2

Discussion

Anti-P-gp agents may improve the sensitivity of chemotherapeutic agents, which can be mediated by P-gp [4]. P-gp inhibitors modified the pharmacokinetic parameters of chemotherapeutic agents, which suggests that P-gp inhibition mediates the metabolism of anticancer drugs [33–37]. However, dofequidar fumarate, a new P-gp inhibitor, was shown to improve the progression-free survival of metastatic breast cancer patients, but it did not modify the AUC of doxorubicin in a study by Saeki et al. [38]. In this study, we investigated the PK parameter of PXL combined with TOR. To avoid the bias of inter-patient variability, we evaluated changes in the PK parameter of PXL in individual patients. There was no significant difference in C_{max} or the AUC of PXL in the presence or absence of TOR. In vitro data indicate that PXL concentrations increase significantly in doxorubicin-resistant MCF-7 cells in the presence of TOR [24]. TOR did not change PXL metabolism in patients, which suggests that concentrations of PXL in malignant cells may increase in vivo. Moreover, PK parameters of TOR in the presence of PXL were similar to those of TOR alone [39], which suggests that there might be no drug–drug interaction between PXL and TOR. TOR might be a substrate for P-gp [40]. However, no differences in the PK parameters of PXL were observed with or without TOR, which suggests that TOR might not change PXL metabolism [41]. In addition, the PK profile of PXL in the presence of TOR was similar to that of PXL alone, which suggests that TOR might not reduce the effect of PXL [42]. In fact, in a phase I trial, PXL had an overall response rate of 24% in patients with metastatic breast cancer who had previously been treated with anthracycline-containing chemotherapy [43]. In our study, the response rate to the PXL + TOR regimen was similar to that with PXL alone. Our PK and phase I data indicate that PXL + TOR might have no negative effects compared to treatment with PXL alone. In addition, our data suggest that there may be no intra-patient variability in the pharmacokinetic parameters of PXL.

Chemoendocrine therapy is one of the recommended treatment options for primary breast cancer and has been shown to be effective in treating breast cancer categorized as "endocrine nonresponsive" or "endocrine response uncertain" by an international consensus panel [18]. However, the optimal combination of drugs and the timing of administration is still being investigated. Whether chemotherapeutic and hormonal agents should be administered sequentially or concurrently is one of the key questions to be answered concerning the treatment of breast cancer patients. A study of SWOG-8814 showed that the sequential use of tamoxifen with cyclophosphamide + doxorubicin + 5-fluorouracil combination chemotherapy may be superior to their concurrent use in terms of overall survival [44]. On the other hand, the concurrent use of tamoxifen and doxorubicin + cyclophosphamide (AC) resulted in a better response rate compared with AC in the treatment of endocrine-therapy-resistant metastatic breast cancer [45]. Concerning drug resistance, the concurrent use of chemotherapeutic and endocrine agents may be reasonable. The number of adverse events observed after PXL alone was not significantly different from the number observed after PXL + TOR. Thus, the combination of PXL and TOR is considered to be safe and tolerable for metastatic breast cancer patients.

The results of this study suggest that the concurrent use of PXL and TOR may be a reasonable treatment option for metastatic breast cancer. Additional clinical trials may be required to clarify the improved efficacy of this chemo-endocrine therapy.

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References

1. Dean M, Rzhetsky A, Allikmets R. The human ATP-binding cassette (ABC) transporter superfamily. Genome Res. 2001;11:1156–66.
2. Cocker HA, Tiffin N, Pritchard-Jones K, et al. In vitro prevention of the emergence of multidrug resistance in a pediatric rhabdomyosarcoma cell line. Clin Cancer Res. 2001;7:3193–8.
3. Gottesman MM, Fojo T, Bates SE. Multidrug resistance in cancer: role of ATP-dependent transporters. Nat Rev Cancer. 2002;2:48–58.
4. Trock BJ, Leonessa F, Clarke R. Multidrug resistance in breast cancer: a meta-analysis of MDR1/gp170 expression and its possible functional significance. J Natl Cancer Inst. 1997;89:917–31.
5. Tsuruo T. Mechanism of multidrug resistance and implications for therapy. Jpn J Cancer Res. 1998;79:285–8.
6. Leonard GD, Fojo T, Bates SE. The role of ABC transporters in clinical practice. Oncologist. 2003;8:411–24.
7. Lagas JS, Vlamning ML, Tellingen OV, et al. Multidrug resistance protein 2 is an important determinant of PXL pharmacokinetics. Clin Cancer Res. 2006;12(20):6125–32.
8. Hoogstraten B, George SL, Samal B, et al. Combination chemotherapy and adriamycin in patients with advanced breast cancer. a Southwest Oncology Group Study. Cancer. 1976;38:13–20.
9. Early Breast Cancer Trialists' Collaborative Group. Polychemotherapy for early breast cancer: an overview of the randomised trials. Lancet. 1998;352:930–42.
10. Fisher B, Redmond C, Legault-Poisson S, et al. Postoperative chemotherapy and tamoxifen compared with tamoxifen alone in the treatment of positive-node breast cancer patients aged 50 years and older with tumors responsive to tamoxifen: results from the National Surgical Adjuvant Breast and Bowel Project B-16. J Clin Oncol. 1990;8:1005–18.
11. Fisher B, Dignam J, Wolmark N, et al. Tamoxifen and chemotherapy for lymph node-negative, estrogen receptor-positive breast cancer. J Natl Cancer Inst. 1997;89:1673–82.
12. Henderson IC, Harris JR. Breast disease. In: Henderson IC, Harris JR, editors. Principles in the management of metastatic disease, 2nd edn. New York: Lippincott; 1990. p. 547–677.
13. Esteva FJ, Valero V, Pusztai L, et al. Chemotherapy of metastatic breast cancer: what to expect in 2001 and beyond. Oncologist. 2001;6:133–46.
14. Pyrhonen S, Valavaara R, Modig H, et al. Comparison of toremifene and tamoxifen in post-menopausal patients with advanced breast cancer: a randomized double blind, the "Nordic" phase III study. Br J Cancer. 1997;76:270–7.
15. Henderson IC, Berry DA, Demetri GD, et al. Improved outcomes from adding PXL but not from escalating doxorubicin in an adjuvant chemotherapy regimen for patients with node-positive primary breast cancer. J Clin Oncol. 2003;15:976–83.
16. Early Breast Cancer Trialists' Collaborative Group. Tamoxifen for early breast cancer: an overview of the randomized trials. Lancet. 1998;351:1451–67.
17. Osborne CK. Tamoxifen in the treatment of breast cancer. N Engl J Med. 1998;339:1609–18.
18. Goldhirsch A, Glick JH, Gelber RD, et al. Meeting highlights: international consensus panel on the treatment of primary breast Cancer. Ann Oncol. 2005;16(10):1569–83.
19. Chevillard S, Pouillart P, Beldjord C, et al. Sequential assessment of multidrug resistance phenotype and measurement of S-phase fraction as predictive markers of breast cancer response to neoadjuvant chemotherapy. Cancer. 1996;77:292–300.
20. Colleoni M, Li S, Gelber RD, et al. Timing of CMF chemotherapy in combination with tamoxifen in postmenopausal women with breast cancer: role of endocrine responsiveness of the tumor. Ann Oncol. 2005;16(5):716–25.
21. Naito M, Yusa K, Tsuruo T. Steroid hormones inhibit binding of Vinca alkaloid to multidrug resistance related P-glycoprotein. Biochem Biophys Res Commun. 1989;158:1066–71.
22. Leonessa F, Clark R. ATP binding cassette transporters and drug resistance in breast cancer. Endocr Relat Cancer. 2003;10:43–73.
23. Clarke R, Currier S, Kaplan O, et al. Effect of P-glycoprotein expression on sensitivity to hormones in MCF-7 human breast cancer cells. J Natl Cancer Inst. 1992;84(19):1458–60.
24. Maruyama S, Kuroiwa S, Saimoto A, et al. Combined effects of toremifene and paclitaxel on human breast cancer cell lines. Jpn J Cancer. 2003;30:669–75.
25. Kuroiwa S, Maruyama S, Okada M, et al. The in vitro combination-effect of toremifene with CAF on growth of various human mammary carcinomas. Jpn J Cancer Chemother. 1998;25:1581–9.
26. Childs S, Yeh RL, Hui D, et al. Taxol resistance mediated by transfection of liver-specific sister gene of P-glycoprotein. Cancer Res. 1998;58:4160–7.
27. Mechetner E, Kyshtoobayeva A, Zonis S, et al. Levels of multidrug resistance (MDR1) P-glycoprotein expression by human breast cancer correlate with in vitro resistance to taxol and doxorubicin. Clin Cancer Res. 1998;4:389–98.
28. Bradshaw DM, Arceci RJ. Clinical relevance of transmembrane drug efflux as a mechanism of multidrug resistance. J Clin Oncol. 1998;16:3674–90.
29. Sikic BI, Fisher GA, Lum BL, et al. Modulation and prevention of multidrug resistance by inhibitors of P-glycoprotein. Can Chemother Pharmacol. 1997;40(Suppl):S13–9.
30. Sparreboom A, van Tellingen O, Nooijen WJ, et al. Tissue distribution, metabolism, and excretion of PXL in mice. Anticancer Drugs. 1996;7:78–86.
31. Sai K, Saito Y, Fukushima-Uesaka H, et al. Impact of CYP3A4 haplotypes on irinotecan pharmacokinetics in Japanese cancer patients. Can Chemother Pharmacol. 2008;62(3):529–37.
32. Berthou F, Dreano Y, Belloc C, Kangas L, et al. Involvement of cytochrome P450 3A enzyme family in the major metabolic pathways of toremifene in human liver microsomes. Biochem Pharmacol. 1994;47(10):1883–95.
33. Sandor V, Fojo T, Bates SE. Future perspectives for the development of P-glycoprotein modulators. Drug Resist Updat. 1998;1(3):190–200.
34. Ambudkar SV, Dey S, Hrycyna CA, et al. Biochemical, cellular, and pharmacological aspects of the multidrug transporter. Annu Rev Pharmacol Toxicol. 1999;39:361–98.
35. Michalak K, Hendrich AB, Wesolowska O, et al. Compounds that modulate multidrug resistance in cancer cells. Cell Biol Mol Lett. 2001;6:362–6.
36. Krishna R, Mayer LD. Multidrug resistance (MDR) in cancer. Mechanisms, reversal using modulators of MDR and the role of MDR modulators in influencing the pharmacokinetics of anticancer drugs. Eur J Pharm Sci. 2000;11:265–83.
37. Cole SP, Sparks KE, Fraser K, et al. Pharmacological characterization of multidrug resistant MRP-transfected human tumor cells. Cancer Res. 1994;54:5902–10.

38. Saeki T, Nomizu T, Toi M, et al. Dofequider fumarate (MS-209) in combination with cyclophosphamide, doxorubicin, and fluorouracil for patients with advanced or recurrent breast cancer. J Clin Oncol. 2007;25:411–7.
39. Tominaga T, Hayashi K, Hayasaka A, et al. Phase I study of NK6222, (toremifene citrate). Jpn J Cancer Chemother. 1992;19(14):2363–72.
40. Keen JC, Miller EP, Bellamy C, et al. P-glycoprotein and resistance to tamoxifen. Lancet. 1994;343:1047–8.
41. Fisher GA, Lum BL, Hausdorff J, et al. Pharmacological considerations in the modulation of multidrug resistance. Eur J Cancer. 1996;32A:1082–8.
42. Sonnichsen DS, Relling MV. Paclitaxel and docetaxel. In: Grochow LB, Ames MM, editors. A clinician's guide to chemotherapy pharmacokinetics and pharmacodynamics. Baltimore, MD: Williams & Wilkins; 1998. p. 375–94.
43. Gianni L, Capri G, Munzone E, et al. Paclitaxel (Taxol) efficacy in patients with advanced breast cancer resistant to anthracyclines. Semin Oncol. 1994;21(Suppl 8):29–33.
44. Albain KS, Green SJ, Ravdin PM, et al. Adjuvant chemohormonal therapy for primary breast cancer should be sequential instead of concurrent: initial results from intergroup trial 0100(SWOG-8814). Proc Am Soc Clin Oncol. 2002;21:143a.
45. Adachi I, Watanabe T, Abe K. Randomized comparison of sequential endocrine and chemotherapy versus combined chemoendocrine therapy in patients with advanced breast cancer. In: Kimura K, Ota K, Yamada K, Saito H, editors. Cancer chemotherapy challenges for the future. New York: Elsevier; 1990. p. 276–87.

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Evaluation of the safety and tolerability of oral TAS-108 in postmenopausal patients with metastatic breast cancer

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Background: The potential of TAS-108 for the treatment of breast cancer has been shown by preclinical studies. We therefore investigated the safe dosage, tolerability, and effectiveness on hormone levels and bone metabolism markers and the pharmacokinetics of TAS-108 administered in postmenopausal Japanese women with metastatic breast cancer.

Patients and methods: The subjects had previously undergone standard endocrine therapeutic modalities. TAS-108 was given repeatedly to five patients each, at three dose levels (40, 80, and 120 mg p.o.) once a day after the first daily meal for a scheduled 8 weeks. Plasma concentrations of TAS-108 and its metabolites were measured at the scheduled time points.

Results: Fifteen patients received TAS-108 treatment. Orally administered TAS-108 was well tolerated at doses up to 120 mg and did not cause notable changes either in hormone levels or bone metabolism markers. Pharmacokinetic results indicated dose-dependent increases in plasma levels of TAS-108 and its metabolites. A steady state was achieved by 2 weeks at all dose levels, suggesting no marked accumulation. Clinical benefits were confirmed in 5 of 15 patients.

Conclusions: Repeated oral administration of TAS-108 at doses up to 120 mg was well tolerated, and the plasma level of this compound increased dose-dependently.

Key words: breast cancer, pharmacokinetics, phase I, postmenopausal, SERM, TAS-108

introduction

Tamoxifen, a selective estrogen receptor modulator (SERM), has been widely used in pre- and postmenopausal women with hormone receptor-positive advanced breast cancer for the past three decades and is also used for adjuvant therapy after surgery for breast cancer [1–4]. Recently, endoxifen, a primary active metabolite of tamoxifen, has been reported to be biotransformed by CYP2D6 enzyme, but that those individuals with breast cancer possessing CYP2D6 polymorphism may have shorter relapse-free survival and time to progression [5, 6]. This suggests the importance of the pharmacokinetic profiles of SERMs.

TAS-108, (7α)-21-[4-[(diethylamino)methyl]-2-methoxyphenoxy]-7-methyl-19- norpregna-1,3,5(10)-trien-3-ol 2-hydroxy-1,2,3-propanetricarboxylate, is a novel steroidal antiestrogen compound mainly metabolized by CYP3A4, which has been shown to bind strongly to estrogen receptor (ER)α and ERβ, with modes of action unlike tamoxifen and fulvestrant in molecular biological studies [7, 8]. Preclinical and animal studies reported that TAS-108 was effective against estrogen-dependent tumors and also against tamoxifen-resistant tumors, with a positive effect on bone mineral density and lower pathological effect on endometrial tissue than tamoxifen [7, 9]. Therefore, TAS-108 may have potential for the treatment of breast cancer.

In the United States, phase I studies have been carried out in healthy postmenopausal women and in postmenopausal women with metastatic breast cancer [10, 11]. TAS-108 was well tolerated up to doses of 160 mg with repeated oral administration. In terms of efficacy, TAS-108 achieved stable disease (SD) at all doses of 40–160 mg, suggesting possible antitumor activity in that range [11]. This study also established that TAS-108 has a linear pharmacokinetic profile with respect to C_{max} [11].

Our study was a first phase I repeated-dose clinical study in Japan involving postmenopausal patients with breast cancer. We sought to establish safe dose levels, tolerability, effects on hormone levels and bone metabolism markers, and the

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pharmacokinetics of TAS-108, in which the doses of TAS-108 (40, 80, and 120 mg) were tested on the basis of tolerability, efficacy, and pharmacokinetic data from prior studies on healthy women and breast cancer patients.

patients and methods

patients and study design

We enrolled 16 postmenopausal patients aged 50–78 years with histologically or cytologically confirmed locally advanced or metastatic breast cancer. The postmenopausal status was defined as being amenorrheic for at least 1 year (except for luteinizing hormone-releasing hormone agonist-induced amenorrhea) or having had a hysterectomy and with both serum 17β-estradiol and follicle-stimulating hormone (FSH) levels in the postmenopausal range. All patients had ER-positive and/or progesterone receptor-positive breast cancer and at least one measurable lesion or bone metastasis. Patients had to have received prior standard endocrine therapy which had been terminated at least 2 weeks before starting the treatment. Prior chemotherapy was allowed if it had been stopped for at least 3 weeks before initiating the present treatment. Additional eligibility requirements included adequate organ function [i.e. leukocyte count ≤10 000/μl, absolute granulocyte count ≥1500/μl, platelet count ≥75 000/μl, hemoglobin ≥9.0 g/dl, total bilirubin and creatinine ≤1.5-fold the upper limit of normal (ULN), aspartate aminotransferase, alanine aminotransferase (ALT) and alkaline phosphatase ≤2.5-fold the ULN (except for patients with liver or bone metastasis who could have ≤5-fold the ULN)]; a predicted life expectancy of ≥3 months; performance status (PS) of two or less on the Zubrod scale.

Patients were ineligible if they had allergy to drugs; past serious thromboembolism; current serious complication(s); active double cancer; lung metastasis with cancer-related lymphangitis and brain metastasis with any symptoms; only one lesion and that lesion had been treated with radiotherapy. Written informed consent was obtained from all patients. The study was approved by the institutional review boards of each participating centre.

In this phase I, open-label, nonrandomized study, patients repeatedly took TAS-108 (in units of 40 mg tablets, Taiho Pharmaceutical Co., Ltd, Tokyo, Japan) orally once a day after the first meal of the day. Eight-week administration was scheduled, but drug treatment was terminated if progression of disease (PD) was observed during the period. After week 8, the drug treatment could be continued if complete response (CR), partial response (PR), or SD had been confirmed. Patients were enrolled in one of three dose groups (40, 80, and 120 mg; five patients per group). If no drug-related grade 3 or more severe adverse event (AE) was observed in the first 14 days of treatment in the first five patients, the enrollment of patients in the next dose level was permitted. If similar serious drug-related AEs were observed in two patients or more, enrollment in the next dose level was to be cancelled.

On each visit to the outpatient clinic, patients were given a physical examination. We evaluated results of laboratory examinations (at baseline and every week), vital signs (at baseline and every 2 weeks), and performed electrocardiography (at baseline and week 2). Hormones [testosterone, FSH, thyroid-stimulating hormone, cortisol, prolactin, estrone, 17β-estradiol, estriol, luteinizing hormone, and sex hormone-binding globulin (SHBG)] and bone metabolism markers [serum osteocalcin and cross-linked carboxy-terminal telopeptide of type I collagen (ICTP)] were assessed at baseline, week 4, and week 8. Endometrial thickness was measured by transvaginal ultrasonography at baseline and week 8. The above examinations were also assessed at the end of the study and at regular intervals when the drug treatment continued >8 weeks.

end points

The primary end point was to investigate the safe dosage and tolerance of TAS-108 administered in postmenopausal Japanese women with metastatic breast cancer subsequent to standard endocrine therapies. In this study, the following secondary end points were also evaluated; effects on hormone levels and bone metabolism markers, tumor assessment, and pharmacokinetics of TAS-108 and its metabolites (deEt-TAS-108, TAS-108-COOH and O-Me-deEt-TAS-108) in human plasma.

safety and efficacy evaluations

Safety assessments were made according to the National Cancer Institute—Common Toxicity Criteria (by Japan Clinical Oncology Group, v2.0). Patients were assessed every 4 weeks using the Response Evaluation Criteria in Solid Tumors criteria after initiation of TAS-108 treatment. Patients with evaluable lesions which were not measurable, such as bone metastasis, were assessed using the General Rules for Clinical and Pathological Recording of Breast Cancer (14th edition) [12]. The clinical benefit rate was determined by the total number of eligible patients who achieved a CR or PR plus those who had SD for at least 24 weeks. CR was defined as the disappearance of all known lesions for at least 4 weeks. PR was defined as at least a 30% decrease in the sum of the longest diameters of all measurable lesions.

pharmacokinetics

On the day of initiation of treatment, blood samples (3 ml) were collected for measurements of TAS-108 and its metabolites in plasma at predose and 4 h after the first administration. In addition, blood samples were obtained before administration of TAS-108 on one day each in weeks 1, 2, 4, and 8 during treatment. Blood samples were collected into ice-cooled sodium heparin tubes. Plasma was obtained by centrifugation (1900 *g*) and then stored at −70°C until analysis. Plasma concentrations of TAS-108 and its metabolites were analyzed with validated methods by liquid chromatographic method with tandem mass spectrometric detection.

results

patient characteristics

Of the 16 patients enrolled, we treated 15 with TAS-108. One patient was ineligible because she had not been off endocrine therapy for at least 2 weeks before entry into the study and therefore did not receive treatment with TAS-108. Equal numbers of the remaining patients were allocated to three different dose groups (40, 80, and 120 mg). All but one patient had recurrent breast cancer (Table 1). There was no patient with only bone metastasis. Fifteen enrolled patients had previously received an average of three endocrine therapy and one chemotherapy regimens. Eleven patients had a PS of zero and the remaining had a PS of one. All patients were ER positive.

safety

Throughout the treatment period, drug-related AEs (definite, probable, possible) were observed in eight patients. They included three grade 2 AEs and 12 grade 1 AEs (Table 2). No grade 3 or more severe AEs were observed. The severity of the AEs was unrelated to the dose level of TAS-108.

There were no notable changes over the course of the study in hormone levels and bone metabolism markers (Figure 1). We measured endometrial thickness ultrasonographically in 13 patients (Figure 1). Of these patients, one in the 80-mg dose group had endometrial hyperplasia after 6 weeks of

Table 1. Patient characteristics at baseline

Characteristics	Number of patients		
	40 mg ($n = 5$)	80 mg ($n = 5$)	120 mg ($n = 5$)
Median age in year (range)	58 (51–61)	66 (55–78)	57 (50–64)
Zubrod performance status			
0	4	3	4
1	1	2	1
2	0	0	0
Diagnosis			
Advanced	0	1	0
Recurrent	5	4	5
Estrogen receptor			
Positive	5	5	5
Negative	0	0	0
Unknown	0	0	0
Progesterone receptor			
Positive	5	4	4
Negative	0	1	1
Unknown	0	0	0
HER2			
0	3	1	2
1+	0	1	0
2+	0	1	0
3+	1	0	0
Unknown	1	2	3
Prior treatment			
Surgery	5	4	5
Endocrine therapy	5	5	5
Regimens for breast cancer			
0	0	0	0
1	0	0	1
2	1	1	1
3	2	2	1
4	1	0	0
5	0	0	1
6	1	1	1
7	0	0	0
8	0	1	0
Chemotherapy	5	3	5
Regimens for breast cancer			
0	0	2	0
1	0	2	3
2	2	0	1
3	1	0	0
4	2	0	1
5	0	1	0
Radiotherapy	3	2	3

Table 2. Drug-related adverse events

Event	Number of patients											
	40 mg ($n = 5$)				80 mg ($n = 5$)				120 mg ($n = 5$)			
	1	2	3	4	1	2	3	4	1	2	3	4
Hot flashes					1				1			
Arthralgia					1					1		
Nausea					1							
Headache									1			
Endometrial hyperplasia					1							
Hyperhidrosis										1		
Musculoskeletal stiffness									1			
Pain in extremity					1							
Alanine aminotoransferase increased					1				2			
Asparate aminotransferase increased									1			
Blood cholesterol increased		1										

administration. This single case had received tamoxifen as adjuvant therapy, and tamoxifen-induced endometrial hyperplasia was observed at the time of relapse. The treatment for recurrence or metastatic disease was sequential treatment of anastrozole and medroxyprogesterone acetate (MPA), and some withdrawal bleeding due to MPA treatment might naturally occur. At the beginning of our study, we performed ultrasonography, but this could not reveal the original baseline thickness before tamoxifen or MPA treatment. The subsequent hyperplasia was considered to be possibly related to TAS-108 intake.

pharmacokinetics

The plasma levels of TAS-108 and its metabolites, deEt-TAS-108, TAS-108-COOH, and O-Me-deEt-TAS-108, increased in a dose-dependent manner, although the mean plasma concentrations of deEt-TAS-108 at a dose of 80 mg at 4 h after the first administration were slightly higher than those receiving a dose of 120 mg due to individual variability (Table 3). Based on the mean minimum concentration profiles of TAS-108 and its metabolites, deEt-TAS-108 and TAS-108-COOH, the steady state was estimated to be achieved by 2 weeks in each dose group, indicating that there was no remarkable accumulation of TAS-108 and its metabolites. The plasma concentration of O-Me-deEt-TAS-108 approximately doubled at week 8 relative to that at week 1 after the first administration at each dose of TAS-108.

efficacy

PR was seen in two patients, SD in nine patients, in three of which, SD findings continued for at least 24 weeks, PD in three patients and not evaluable in one patient. One patient each in the 40-mg and 120-mg dose groups showed PR, while two patients in the 40-mg dose group and one patient in the 80-mg dose group showed SD for at least 24 weeks (Table 4). The overall clinical benefit rate for all dosages was 33.3%.

discussion

We set out to determine whether repeated oral administration of TAS-108 at the dose levels tested in this study was well tolerated with efficacy in postmenopausal Japanese patients

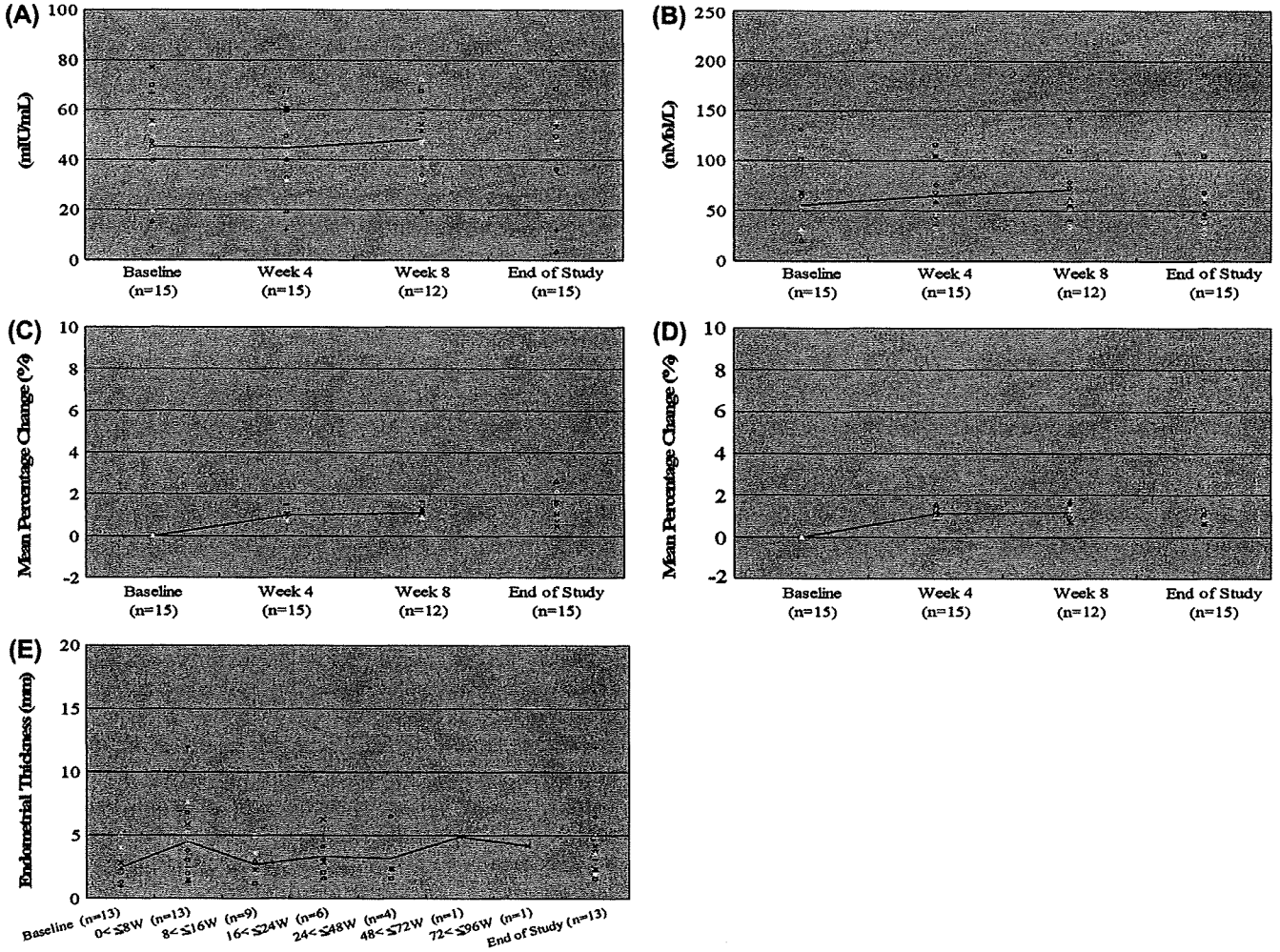

Figure 1. Individual and mean plasma concentration-period profiles [(A) FSH and (B) SHBG], relative mean changes-period profiles [(C) I CTP and (D) osteocalcin] and endometrial thickness -period profiles (E) of all patients.

Table 3. Individual concentrations of TAS-108 and its metabolites in human plasma[a]

TAS-108	40	0.0000 (0.0000)	3.036 (1.064)	0.2076 (0.1244)	0.2905 (0.2463)	0.3055 (0.1589)	0.3055 (0.1720)
	80	0.0000 (0.0000)	6.058 (1.619)	0.6055 (0.1122)	0.7754 (0.2272)	0.7163 (0.1677)	0.9912 (0.5416)
	120	0.0000 (0.0000)	10.72 (7.21)	1.826 (1.341)	1.664 (1.008)	1.446 (0.371)	2.199 (1.688)
deEt-TAS-108	40	0.0000 (0.0000)	0.7684 (0.2589)	0.3222 (0.1562)	0.3735 (0.1996)	0.3625 (0.1989)	0.4438 (0.1556)
	80	0.0000 (0.0000)	2.164 (1.347)	0.8218 (0.2683)	0.9574 (0.3628)	0.9814 (0.2798)	1.560 (0.7183)
	120	0.0000 (0.0000)	2.160 (1.833)	1.434 (0.828)	1.447 (0.653)	1.244 (0.320)	1.762 (0.763)
TAS-108-COOH	40	0.0000 (0.0000)	11.69 (8.52)	9.400 (6.718)	9.343 (7.152)	9.561 (9.889)	11.99 (10.56)
	80	0.0000 (0.0000)	20.84 (8.75)	26.99 (15.38)	26.52 (22.30)	27.74 (19.55)	43.26 (27.04)
	120	0.0000 (0.0000)	49.73 (29.31)	33.33 (23.52)	39.81 (40.25)	26.22 (11.89)	35.90 (18.98)
O-Me-deEt-TAS-108	40	0.0000 (0.0000)	0.4643 (0.2703)	5.347 (1.935)	8.105 (3.193)	10.27 (4.07)	11.90 (4.21)
	80	0.0000 (0.0000)	1.254 (0.412)	11.43 (2.80)	17.28 (4.52)	20.63 (4.09)	27.34 (7.19)
	120	0.0000 (0.0000)	1.402 (1.045)	16.14 (4.78)	21.43 (6.29)	26.49 (6.46)	34.41 (11.57)

All data represent mean values (SD). Below the quantitation limit were presumed to be 0.0000 ng/ml. The quantitation limit was 0.1000 ng/ml for TAS-108, deEt-TAS-108 and O-Me-deEt-TAS-108 and 0.5000 ng/ml for TAS-108-COOH.

[a] $n = 5$ patients for each group except for the number of patients at 8 weeks ($n = 3$).

Table 4. Response to TAS-108 treatment

Best overall tumor response	Number of patients		
	40 mg ($n = 5$)	80 mg ($n = 5$)	120 mg ($n = 5$)
CR	0	0	0
PR	1	0	1
SD ≥24 weeks	2	1	0
SD	1	1	4
PD	1	2	0
NE	0	1	0

CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; NE, not evaluable.

with metastatic breast cancer that had progressed subsequent to previous standard endocrine therapy.

In terms of safety, eight patients had mild (grades 1–2) drug-related AEs, mainly involving hot flashes, arthralgia, and ALT increase. There was no grade 3 or more severe drug-related AE. Blakely et al. [11] reported the pattern of AEs in a phase I study of TAS-108 in which the major AEs included hot flashes, headache, and nausea, which were similar to those associated with other SERMs such as tamoxifen. The pattern of AEs observed in this study was also similar to that in the report by Blakely et al. i.e. there was with no relationship to dose. TAS-108 did not cause notable changes of hormone levels (such as FSH, SHBG), while it was reported that tamoxifen caused those changes in the clinical trials [13]. This observation suggests that TAS-108 may have lower estrogenic effects on hypothalamus–pituitary axis and on the liver, unlike tamoxifen. In addition, thromboembolic events associated with tamoxifen treatment [14, 15] were not seen in this study. Aromatase inhibitors (AIs) have shown improved efficacy over tamoxifen and are recommended as both first-line therapy for advanced breast cancer and in the adjuvant setting for the treatment of early breast cancer [16–18]. However, breast cancer patients receiving AIs have a higher incidence of osteoporosis, bone fracture, and arthralgia, which can result in discontinuation of treatment [19, 20]. Bone toxicity was not seen in this study which likely reflects the positive effects on bone metabolism reported in animal study [9]. Furthermore, remarkable changes in bone metabolism markers were not seen. The arthralgia experienced by 2 of 15 patients was not clinically significant; the patients had mild symptoms (grade 1 or 2) and soon recovered without any additional medications. Endometrial hyperplasia was recorded in one patient as drug-related AE, but it is unclear whether the event was completely attributable to TAS-108 considering the effects of previous treatments with tamoxifen and MPA on her endometrium (see 'Results'). It has been reported that tamoxifen has unfavorable endometrial effects in Caucasian and Japanese patients. On the other hand, TAS-108 was reported to have no effect on the endometrial lining in the previous clinical study by Blakely et al. [11] and had lower uterotrophic effects than tamoxifen in an animal study [7, 9]. Therefore, TAS-108 was anticipated to have a low risk of endometrial hyperplasia. However, further study is necessary to determine the effect of the long-term use of TAS-108 treatment on the endometrium in a greater number of cases.

In pharmacokinetics, the plasma concentration of TAS-108 at 4 h following administration after food intake in this study was similar to the C_{max} of TAS-108 in Caucasian patients; therefore, it was suggested that the results of exposure to TAS-108 in Japanese patients were not extremely different from that in Caucasian patients. Based on the mean time–minimum concentration profiles of TAS-108 and its metabolites after repeated TAS-108 administration in each dose group in the present study, a steady state was estimated in the pharmacokinetics of TAS-108 to be achieved by 2 weeks, suggesting no remarkable accumulation of TAS-108 and its metabolites.

Two patients achieved PR in the 40- and 120-mg dose groups, and three patients achieved SD for at least 24 weeks in the 40- and 80-mg dose group. Although the sample size of this study was not large enough to establish dose response, TAS-108 had antitumor activity with a total clinical benefit rate of 33.3% in patients who had been heavily treated previously with an average of three endocrine therapy and one chemotherapy regimen. In addition, the majority of patients had received tamoxifen therapy which also had failed. There were 13 patients who had received tamoxifen in the adjuvant treatment and/or treatment for advanced or recurrent breast cancer, and it was encouraging that two achieved PR and two SD for at least 24 weeks.

TAS-108 at all dose levels was well tolerated with no unacceptable toxicity and had antitumor activity in postmenopausal Japanese patients with metastatic breast cancer. A multiinstitutional phase II study to identify the optimal dose of TAS-108 in postmenopausal Japanese women with metastatic breast cancer is in progress.

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references

1. Osborne CK. Tamoxifen in the treatment of breast cancer. New Engl J Med 1998; 339: 1609–1618.
2. Early Breast Cancer Trialists' Collaborative Group. Tamoxifen for early breast cancer: an overview of the randomised trials. Lancet 1998; 351: 1451–1467.
3. Clemons M, Danson S, Howell A. Tamoxifen ('Nolvadex'): a review. Cancer Treat Rev 2002; 28: 165–180.

4. Jaiyesimi IA, Buzdar AU, Decker DA et al. Use of tamoxifen for breast cancer: twenty-eight years later. J Clin Oncol 1995; 13: 513–529.
5. Goetz MP, Knox SK, Suman VJ et al. The impact of cytochrome P450 2D6 metabolism in women receiving adjuvant tamoxifen. Breast Cancer Res Treat 2007; 101: 113–121.
6. Lim HS, Lee HJ, Lee KS et al. Clinical Implications of CYP2D6 genotypes predictive of tamoxifen pharmacokinetics in metastatic breast cancer. J Clin Oncol 2007; 25: 3837–3845.
7. Yamamoto Y, Shibata J, Yonekura K et al. TAS-108, a novel oral steroidal antiestrogenic agent, is a pure antagonist on estrogen receptor α and a partial agonist on estrogen receptor β with low uterotrophic effect. Clin Cancer Res 2005; 11: 315–322.
8. Yamamoto Y, Wada O, Takada I et al. Both N- and C-terminal transactivation functions of DNA-bound ERα are blocked by a novel synthetic estrogen ligand. Biochem Biophys Res Commun 2003; 312: 656–662.
9. Toko T, Shibata J, Sato K et al. Antiestrogenic/estrogenic activities of TAS-108 (SR16234), a new steroidal selective estrogen receptor modulator. Breast Cancer Res Treat 1999; 57: 52.
10. Yamaya H, Yoshida K, Kuritani J et al. Safety, tolerability, and pharmacokinetics of TAS-108 in normal healthy post-menopausal female subjects: a phase I study on single oral dose. J Clin Pharm Ther 2005; 30: 459–470.
11. Blakely LJ, Buzdar A, Chang HY et al. A phase I and pharmacokinetic study of TAS-108 in postmenopausal female patients with locally advanced, locally recurrent inoperable, or progressive metastatic breast cancer. Clin Cancer Res 2004; 10: 5425–5431.
12. The Japanese Breast Cancer Society. General Rules for Clinical and Pathological Recording of Breast Cancer, Edition 14. Tokyo: Kanehara Public Co., 2000.
13. Ellmen J, Hakulinen P, Partanen A et al. Estrogenic effects of toremifene and tamoxifen in postmenopausal breast cancer patients. Breast Cancer Res Treat 2003; 82: 103–111.
14. Fisher B, Costantino JP, Wickerham DL et al. Tamoxifen for the prevention of breast cancer: current status of the National Surgical Adjuvant Breast and Bowel Project P-1 study. J Natl Cancer Inst 2005; 97: 1652–1662.
15. Fisher B, Dignam J, Bryant J et al. Five versus more than five years of tamoxifen therapy for breast cancer patients with negative lymph nodes and estrogen receptor-positive tumors. J Natl Cancer Inst 1996; 88: 1529–1542.
16. Brueggemeier RW, Hackett JC, Diaz-Cruz ES. Aromatase inhibitors in the treatment of breast cancer. Endocr Rev 2005; 26: 331–345.
17. Smith IE, Dowsett M. Aromatase inhibitors in breast cancer. N Engl J Med 2003; 348: 2431–2442.
18. Mouridsen HT, Robert NJ. The role of aromatase inhibitors as adjuvant therapy for early breast cancer in postmenopausal women. Eur J Cancer 2005; 41: 1678–1689.
19. Crew KD, Greenlee H, Capodice J et al. Prevalence of joint symptoms in postmenopausal women taking aromatase inhibitors for early-stage breast cancer. J Clin Oncol 2007; 25: 3877–3883.
20. Burstein HJ. Aromatase inhibitor-associated arthralgia syndrome. Breast 2007; 16: 223–234.

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Review

Physiological and Oncogenic Aurora-A Pathway

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Abstract

Aurora family of protein kinases have emerged as crucial factors of, not only mitosis and cytokinesis, but also human carcinogenesis. Among these family members is Aurora-A that is frequently overexpressed in varieties of human cancer. Both in vitro and in vivo studies demonstrated that Aurora-A induces tumorigenesis through genome instability. These studies have further shown that cell signaling cross-talk between Aurora-A and other cellular proteins are essential for fully-transformed phenotypes. This review summarizes recent progress of Aurora-A-associated carcinogenesis.

Key words: Aurora-A, Plk1, mTOR, Cell Cycle, Checkpoint, Genome Instability, Phosphorylation

Introduction

Aurora-A was discovered in a screen for Drosophila mutations affecting the poles of the mitotic spindle function [1]. Transcription of the Aurora-A gene is cell-cycle regulated. Thus, the promoters of the Aurora-A gene contain specific elements (CDE/CHR sequences), which are responsible for transcription at G2 phase of the cell cycle [2-4]. It has been well documented that activation of Aurora-A is required for mitotic entry, centrosome maturation and separation, and G2 to M transition [5.6]. Interestingly, overexpression of Aurora-A is frequently observed in varieties of human cancer, including breast, colorectal, bladder, pancreatic, gastric, ovarian and esophageal cancer [7-12]. Overexpression of Aurora-A in fibroblasts resulted in cell transformation, supporting a notion that high levels of this protein are correlated to cell malignancy [13].

Potential roles of Aurora-A in cell transformation were also demonstrated from recent studies that this kinase phosphorylates a breast cancer tumor suppressor BRCA1 at Ser308 [14]. Both proteins are localized on centrosome at the beginning of mitosis [15], suggesting that signaling between these two proteins are crucial for regulation of normal cell cycle.

Recent studies added a couple of new insight of how Aurora-A induces cell transformation. Thus, in physiological conditions, Aurora-A and its activator collaborate with Plk1, Polo-like kinase 1, to initiate mitosis. On the other hand, in cells transformed with Aurora-A, mTOR pathway is activated [16,17].

In this review, differential roles of Aurora-A in cell cycle and cell transformation are discussed.

Aurora-A and BRCA1

The *Aurora-A* gene locus is located in the 20q13 chromosome region, which is frequently amplified in several different types of malignancies such as breast, colorectal, pancreatic, and bladder cancers [7-12]. In particular, 20q11-q13 regions are amplified in 40% of breast cancer cell lines as well as in 12-18% of primary tumors. Aurora-A protein is a member of the Ser/Thr kinase family, and recent studies have shown that the protein is involved in the G2-M checkpoint and commitment to mitosis [18-21]. Furthermore, it has been demonstrated that Aurora-A is inactivated by DNA damage at the end of the G2 phase, and overexpres-

sion of Aurora-A abrogates the G2 checkpoint, resulting in the amplified centrosome and cell transformation [18]. Significantly, Aurora-A is recruited to the centrosome early in the G2 phase and becomes phosphorylated and activated in the centrosome late in the G2 phase [6].

Deng's lab demonstrated that ~25% of mouse embryonic fibroblasts (MEFs) derived from the *BRCA1* exon 11-deleted mice contains more than two centrosomes, leading to loss of the G2-M checkpoint and aneuploidy [21]. In addition, we and others found that BRCA1 is localized in the centrosome and binds to γ-tubulin [15,22,23].

From these observations, we discovered that BRCA1 functionally interacts with Aurora-A [14]. Interestingly, the aa1314-1863 region of BRCA1 was found to bind to Aurora-A directly. Mutagenic analysis and phospho-specific antibodies revealed that S308 of BRCA1 is normally phosphorylated by Aurora-A early in the M phase. Phosphorylation of BRCA1 S308 by Aurora-A was abolished by treating cells with ionizing radiation. Most interestingly, re-expression of the phospho-deficient form of BRCA1, S308N (N=Asn), in BRCA1-mutated MEFs resulted in growth arrest at the G2 phase without any cell stress, indicating that phosphorylation of BRCA1 S308 is necessary for the transition from G2 to M. These results indicate that an unphosphorylated form of BRCA1 at S308 is necessary for G2-M checkpoint. These are the first indications of the roles of the physiological levels of BRCA1 phosphorylation in regulating the cell cycle. Additional evidence of BRCA1/Aurora-A interaction is that Aurora-A regulates inhibition of centrosome microtubule nucleation mediated by BRCA1's E3 ligase activity [24].

Exogenous overexpression of Aurora-A in human cell culture was further studied by transfecting U2OS osteosarcome cell line [17]. Interestingly, in those cells, increased phosphorylation of BRCA1 S308 was not detected [unpublished results]. These results suggest that phosphorylation of BRCA1 S308 may not be necessary for cell transformation. Thus, perhaps there is substrate selectivity by Aurora-A in physiological and malignant conditions.

Aurora-A and mTOR

Most prominent discoveries from MMTV-Aurora-A transgenic mice are constitutive phosphorylation of mTOR Ser2448 and Akt Ser473 in developed mammary tumors [16]. Mammalian target of rapamycin (mTOR) is a protein serine/threonine kinase that controls a broad range of cellular processes. mTOR exists in two distinct complexes; mTOR complex 1 (mTORC1) and complex 2 (mTORC2). mTOR is phosphorylated at multiple sites, including Ser2448, Ser2481, Thr2446 and Ser1261. Phosphorylation at Ser2448 is mediated by p70 ribosomal S6 kinase (S6K) and occurs predominantly to mTOR in mTORC1 [25-27]. mTORC1 is composed of mTOR, mLST8, raptor and PRAS40. Its function is involved in many growth-related processes such as translation, ribosome biogenesis, transcription, autophagy and hypoxic adaptation, and is sensitive to rapamycin. mTORC2 shares both mTOR and mLST8 with mTORC1. Other unique components in mTORC2 are rapamycin-insensitive companion of mTOR (rictor), mammalian stress-activated protein kinase-interacting protein 1(mSIN1) and proline-rich repeat protein-5 (PRR5) or PRR5-like [28-33].

Two major functions have been ascribed to mTORC2, including regulation of Akt and cell cycle-dependent organization of actin cytoskeleton. mTORC2 phosphorylates Akt at Ser473 in its C-terminal hydrophobic motif, which, in conjunction with PDK1-mediated phosphorylation of Thr308, confers full activation of Akt [34]. mTORC2 regulates actin cytoskeleton through a mechanism that involves the small GTPases Rho and Rac, although the molecular details are largely still unclear [8,35]. Interestingly, mTORC2 phosphorylates PKC and SGK1 (serum- and glucocorticoid-induced protein kinase 1), and has been implicated in controlling cell size [36-39].

Elevated phosphorylation of mTOR Ser2448 and Akt Ser473 in Aurora-A transformed cells suggests that Aurora-A can potentially regulate two mTOR pathways, mTORC1 and mTORC2. Since chemical inhibitors of mTOR can abolish transformed phenotypes induced by Aurora-A [17], it is likely that either or both of mTORC1 and 2 is important for Aurora-A transformation.

Of note, mammary tumor development can be observed only after long latency in MMTV-Aurora-A mice [16]. In cell culture system of stable transfectants, cells in early passage numbers do not contain phosphorylated mTOR and Akt, but cells after long passage numbers they show up [17]. As one possible interpretation, overexpression of Aurora-A is not a strong driving force, but some additional events need to happen to accelerate Aurora-A's tumor development. When mTOR pathway is activated under this situation, cells now acquire the full-transforming ability.

Aurora-A and Plk1

Expression of Plk1 is cell cycle-dependent. Levels of the protein increases in late G2 phase, and decreases during mitotic exit [40]. Kinase activity well

correlates with levels of the protein, thus it increases at G2/M transition and reaches at the maximal during mitosis. Similar to Plk1, levels of Aurora-A increase during G2 and reach at the maximal in early mitosis [13,41]. 'Activator' proteins for Aurora-A have been identified. Those include TPX2, Ajuba, PAK1, HEF1 and hBora [6,42-47]. Among these Aurora-A interactors, hBora expression peaks during G2 and decreases rapidly during mitosis [48,49]. It has also been shown that hBora forms a complex with Plk1 in G2 phase [48,50,51]. Aurora-A's binding to Bora and its subsequent phosphorylation are required for full activation of Aurora-A. In addition, both proteins are essential for Plk1 activation at the centrosome in G2 phase. In this model, it is thought that Bora binding to Plk1 induces allosteric effects that allow Aurora-A to the Plk1 T-loop of its kinase domain, where Aurora-A phosphorylates Thr210, leading to full activation of Plk1 [51,52].

It has been speculated that Aurora-A is a target for ubiquitination by CHFR, checkpoint with FHA and RING finger domains. CHFR regulates an early mitotic checkpoint, during prophase, in response to the disruption of microtubule formation or stabilization as assessed after treatment with microtubule inhibitors such as nocodazole, colcemid and taxanes [53]. Interestingly, Aurora-A was overexpressed in CHFR-null mouse embryonic fibroblasts and tissues, strongly supporting that CHFR ubiquitinates Aurora-A [54]. These studies have also demonstrated that the C-terminal cysteine-rich region of CHFR protein interacts with the N-terminus of Aurora-A protein. Similar results were shown from the other studies that siRNA-mediated depletion of CHFR in MCF10A cells resulted in overexpression of Aurora-A [55]. It has been demonstrated that, in HCT116 cells overexpressing CHFR, there was no change in levels of Aurora-A and localization of Aurora-A to the centrosomes, however, nocodazole-induced CHFR-mediated mitotic delay was associated with unphosphorylation of Aurora-A at Thr288 [56].

Studies of CHFR protein further supported functional interaction between Aurora-A and Plk1. It has been shown that overexpression of CHFR mutants which mimic unphosphorylated CHFR can decrease levels and kinase activity of Plk1 [57]. Interestingly, mouse embryonic fibroblasts from CHFR knockout mice express high levels of Plk1, suggesting that CHFR can ubiquitinate Plk1 to target it for degradation [54].

CONCLUSION

Given the high frequency of overexpression of Aurora-A in human cancers, inhibition of Aurora-A with small compounds looks like an attractive cancer-therapeutic strategy. Several compounds have been synthesized and are under clinical trials.

Classical cell biology assay, such as transfection of normal fibroblasts with Aurora-A cDNA, resulted in cell transformation. Transgenic model targeting Aurora-A in mammary glands also support a notion that this kinase is oncogenic. However, quite long latency and low incidence of tumor development in these mice suggest that Aurora-A alone is not a strong driving force of malignancy, but other hits need to occur for full transformation [16]. Thus, it is possible that inhibition of Aurora-A with compounds may not be sufficient for killing Aurora cancer cells. Chromosome instabilities observed in those mammary tumors support this hypothesis that activation or inactivation of 'effector proteins' due to the gross alteration of chromosome structure may result in accelerating tumorigenesis (Fig. 1). In that sense, simultaneous inhibition of this pathway(s) as well as Aurora-A might be necessary for the better treatment of patients. For example, mTOR/Akt pathway might be the one which is crucial for Aurora-A tumorigenesis.

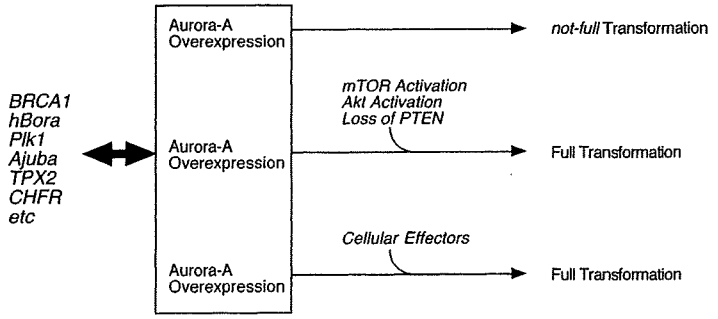

Figure 1. Model of Aurora-A cell transformation. Physiological regulation of Aurora-A kinase activity is by BRCA1, hBora, Ajuba, TPX2 an dPlk1 etc, however, cell transformation by Aurora-A requires additional oncogenic events, such as constitutive activation of mTOR/Akt pathway and loss of PTEN tumor suppressor [17].

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Conflict of Interest

The authors have declared that no conflict of interest exists.

References

1. Glover G.M., et al. Mutations in aurora prevent centrosome separation leading to the formation of monopolar spindles. Cell, 1995. 81: 95-105.
2. Kimura M., et al. Cell cycle-dependent expression and spindle pole localization of a novel human protein kinase, Aik, related to Aurora of Drosophila and yeast Ipl1. J. Bio. Chem., 1997. 272: 13766-13771.
3. Tanaka M., et al. Cell-cycle-dependent regulation of human Aurora A transcription is mediated by periodic repression of E4TF1. J. Biol. Chem., 2002. 277: 10719-10726.
4. Kimura M., et al. Cell-cycle-dependent regulation of the human Aurora B promoter. Biochem. Biophys. Res. Commun., 2004. 316: 930-936.
5. Fu J., et al. Roles of Aurora kinases in mitosis and tumorigenesis. Mol Cancer Res., 2007. 5: 1-10.
6. Hirota T., et al. Aurora-A and an interacting activator, the LIM protein Ajuba, are required for mitotic commitment in human cells. Cell, 2003. 114: 585-598.
7. Tanaka T., et al. Centrosomal kinase AIK1 is overexpressed in invasive ductal carcinoma of the breast. Cancer Res, 1999. 59: 2041-2044.
8. Nishida N., et al. High copy amplification of the Aurora-a gene is associated with chromosomal instability phenotype in human colorectal cancers. Cancer Biol Ther., 2007. 6: 525-533.
9. Compérat E., et al. Aurora-A/STK-15 is a predictive factor for recurrent behavior in non-invasive bladder carcinoma: a study of 128 cases of non-invasive neoplasms. Virchows Arch., 2007. 450: 419-424.
10. Li D., et al. Overexpression of oncogenic STK15/BTAK/Aurora A kinase in human pancreatic cancer. Clin Cancer Res., 2003. 9: 991-997.
11. Lassmann S., et al. Predictive value of Aurora-A/STK15 expression for late stage epithelial ovarian cancer patients treated by adjuvant chemotherapy. Clin Cancer Res., 2007. 13: 4083-4091.
12. Tong T., et al. Overexpression of Aurora-A contributes to malignant development of human esophageal squamous cell carcinoma. Clin Cancer Res., 2004. 10: 7304-7310.
13. Bischoff J.R., et al. A homologue of Drosophila aurora kinase is oncogenic and amplified in human colorectal cancers. EMBO J., 1998. 17: 3052-3065.
14. Ouchi M., et al. BRCA1 phosphorylation by Aurora-a in the regulation of G2 to M transition. J Biol Chem., 2004. 279: 19643-19648.
15. Okada S, and Ouchi T. Cell cycle differences in DNA damage-induced BRCA1 phosphorylation affects its subcellular localization. J Biol Chem., 2003. 278: 2015-2020.
16. Wang X., et al. Overexpression of aurora kinase A in mouse mammary epithelium induces genetic instability preceding mammary tumor formation. Oncogene, 2006. 25: 7148-7158.
17. Taga M., et al. Essential Roles of mTOR/Akt Pathway in Aurora-A Cell Transformation. Int J Biol Sci., 2009. 19: 444-450.
18. Marumoto T., et al. Roles of aurora-a kinase in mitotic entry and G2 checkpoint in mammalian cells. Genes Cell, 2002. 7: 1173-1182.
19. Meraldi P., et al. Aurora-A overexpression reveals tetraploidization as a major route to centrosome amplification in p53-/- cells. EMBO J., 2002. 21: 483-492.
20. Anand S., et al. Aurora-A amplification overrides the mitotic spindle assembly checkpoint, inducing resistance to taxol. Cancer Cell, 2003. 3: 51-62.
21. Xu X., et al. Centrosome amplification and a defective G2-M cell cycle checkpoint induce genetic instability in BRCA1 exon 11 isoform-deficient cells. Mol Cell, 1999. 3: 389-395.
22. Hsu L.C, and White R.L. BRCA1 is associated with the centrosome during mitosis. Proc Natl Acad Sci USA, 1998. 95: 12983-12988.
23. Hsu L.C, et al. Identification of a gamma-tubulin-bondong domain of BRCA1. Cancer Res., 2001. 61: 7713-7718.
24. Sankaran S., et al. Aurora-A kinase regulates breast cancer-associated gene 1 inhibition of centrosome-dependent microtubule nucleation. Cancer Res. 2007. 67: 11186-11194.
25. Chiang G.G., et al. Phosphorylation of mammalian target of rapamycin (mTOR) at Ser-2448 is mediated by p70S6 kinase. J Biol Chem., 2005. 280: 25485-25490.
26. Holz M.K, and Blenis J. Identification of S6 kinase 1 as a novel mammalian target of rapamycin (mTOR)-phosphorylating kinase. J Biol Chem., 2005. 280: 26089-26093.
27. Copp J., et al. TORC-specific phosphorylation of mammalian target of rapamycin (mTOR): phospho-Ser2481 is a marker for intact mTOR signaling complex 2. Cancer Res., 2009. 69: 1821-1827.
28. Jacinto E., et al. SIN1/MIP1 maintains rictor-mTOR complex integrity and regulates Akt phosphorylation and substrate specificity. Cell, 2006. 127: 125-137.
29. Pearce L.R., et al. Identification of protor as a novel rictor-binding component of mTOR complex-2. Biochem J., 2007. 405: 513-522.
30. Sabassov D.D., et al. Rictor, a novel binding partner of mTOR, defines a rapamycin-insensitive and raptor-independent pathway tha regulates the cytoskeleton. Curr Bio., 2004. 14: 1296-1302.
31. Thedieck K., et al. PRAS40 and PRR5-like protein are new mTOR interactors that regulate apoptosis. PLoS ONE, 2007. 2: 1217.
32. Woo S.Y., et al. PRR5, a novel component of mTOR complex 2, regulates platelet-derived growth factor receptor beta expression and signaling. J Biol Chem., 2007. 282: 25604-25612.
33. Yang Q., et al. Identification of Sin1 as an essential TORC2 component required for complex formation and kinase activity. Genes Dev., 2006. 20: 2820-2832.
34. Sarbassov D.D., et al. Phosphorylation and regulation of Akt/PKB by the rictor-mTOR complex. Science, 2005. 307: 1098-1101.
35. Jacinto E., et al. Mammalian TOR complex 2 controls the actin cytoskeleton and is rapamycin insensitive. Nat Cell Biol., 2004. 6: 1122-1128.
36. Facchinetti V., et al. The mammalian target of rapamycin complex 2 controls folding and stability of Akt and protein kinase C. EMBO J., 2008. 27: 1932-1943.
37. Ikenoue T., et al. Essential function of TORC2 in PKC and Akt turn motif phosphorylation, maturation and signaling. EMBO J., 2008. 27: 1919-1931.
38. Garcia-Martinez J.M., and Alessi D.R. mTOR complex 2 (mTORC2) controls hydrophobic motif phosphorylation and

activation of serum- and glucocorticoid-induced protein kinase 1 (SGK1). Biochem J., 2008. 416: 375-385.
39. Rosner M., et al. Functional interaction of mTOR complexes in regulating mammalian cell size and cell cycle. Hum Mol Genet., 2009. 18: 3298-3310.
40. Petronczki M., et al. Polo on the Rise-from mitotic entry to cytokinesis with Plk1. Dev Cell, 2008. 14: 646-659.
41. Barr A.R, and Gergely F. Aurora-A: the maker and breaker of spindle poles. J Cell Sci., 2007. 120: 2987-2996.
42. Wittmann T., et al. TPX2, a novel xenopus MAP involved in spindle pole organization. J Cell Biol., 2000. 149: 1405-1418.
43. Kufer T.A., et al. Human TPX2 is required for targeting Aurora-A kinase to the spindle. J Cell Biol., 2002. 158: 617-623.
44. Eyers P.A., et al. A novel mechanism for activation of the protein kinase Aurora A. Curr Biol., 2003. 13: 691-697.
45. Bayliss R., et al. Structural basis of Aurora-A activation by TPX2 at the mitotic spindle. Mol Cell, 2003. 12: 851-862.
46. Zhao Z.S., et al. The GIT-associated kinase PAK targets to the centrosome and regulates Aurora-A. Moll Cell, 2005. 20: 237-249.
47. Pugacheva E.N., et al. HEF1-dependent Aurora A activation induces disassembly of the primary cilium. Cell, 2007. 129: 1351-1363.
48. Chan E.H., et al. PLK1 regulates mitotic Aurora A function through βTrCP-dependent degradation of hBora. Chromosoma, 2008. 117: 457-469.
49. Seki A., et al. Plk1- and bTrCP-dependent degradation of Bora controls mitotic progression. J Cell Biol., 2008. 181: 65-78.
50. Macurek L., et al. Polo-like kinase-1 is activated by Aurora A to promote checkpoint recovery. Nature, 2008. 455: 119-123.
51. Seki A., et al. Bora and the kinase Aurora A cooperatively activate the kinase Plk1 and control mitotic entry. Science, 2008 320: 1655-1658.
52. Macurek L, et al. Aurora-A and hBora jointhe game of polo. Cancer Res. 2009. 69: 4555-4558.
53. Scolnick D.M, and Halazonetis T.D. Chfr defines a mitotic stress checkpoint that delays entry into metaphase. Nature, 2000. 406: 430-435.
54. Yu X., et al. Chfr is required for tumor suppression and Aurora A regulation. Nat Genet., 2005. 37: 401-406.
55. Privette L.M., et al. Loss of CHFR expression in mammary epithelial cells causes genomic instability by disrupting the mitotic spindle assembly checkpoint. Neoplasia, 2008. 10: 643-652.
56. Summers M.K., et al. The CHFR mitotic checkpoint protein delays cell cycle progression by excluding cyclin B1 from the nucleus. Oncogene, 2005. 24: 2589-2598.
57. Shtivelman E. Promotion of mitosis by activated protein kinase B after DNA damage involves polo-like kinase 1 and checkpoint protein CHFR. Mol Cancer Res., 2003. 1: 959-969.

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Original Article

Efficacy and Tolerability of Weekly Paclitaxel in Combination with High-dose Toremifene Citrate in Patients with Metastatic Breast Cancer

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Toremifene citrate is expected to prevent drug resistance in cancer patients by inhibiting p-glycoprotein activity. The safety and efficacy of combination therapy with high-dose toremifene citrate and paclitaxel were investigated. Between December 2003 and June 2004, 15 women with a mean age of 53 years old with metastatic breast cancer were enrolled. The administration schedule was 80 mg/m^2 of paclitaxel given on Days 1, 8, and 15, and 120 mg/day of toremifene citrate orally administered starting on Day 18. On Days 32 and 39, paclitaxel was concurrently administered again. Toxicities, response rate, and time to treatment failure were assessed. All patients had been treated with endocrine or chemotherapy. Grade 3 leukopenia occurred in 2 patients on the administration of paclitaxel alone, and grade 3 febrile neutropenia occurred in 1 patient given the combination therapy. There was no grade 3 or greater non-hematological toxicity. There was no complete response and 1 partial response, producing a response rate of 6.7%. Median time to treatment failure was 2.7 months. Combination therapy of paclitaxel and toremifene was safe and well tolerated with minimal toxicity. Further clinical trials targeting patients with functional p-glycoprotein are warranted.

Key words: toremifene, paclitaxel, p-glycoprotein, metastatic breast cancer

Metastatic breast cancer is considered incurable and optimal palliation and prolongation of life rather than curative intent are the main goals of treatment [1, 2]. Anthracycline-containing regimens have been the most effective against this disease [3] and until recently, there was no standard treatment for patients with metastatic breast cancer in whom an anthracycline-containing regimen was ineffective. However, taxanes have proved to be equally as efficacious as anthracycline [4], and anthracycline and taxanes are now considered the most active chemotherapeutic agents for metastatic breast cancer [5]. Taxanes have also demonstrated significant activity as second- and third-line agents in the treatment of meta-

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static breast cancer [4, 6]. However, tumors initially sensitive to agents often acquire a multidrug resistance (MDR) phenotype, which is characterized by cross resistance to drugs to which the tumor has not been exposed [7]. A number of mechanisms have been identified for the resistance to chemotherapeutic agents. As one form of resistance, p-glycoprotein encoded by *MDR1* as an energy-dependent drug efflux pump can acquire resistance to structurally unrelated compounds simultaneously [8]. Toremifene citrate was developed in the 1980s, as a safe, less toxic, and non-steroidal triphenylethylene antiestrogen and became widely used in the treatment of postmenopausal breast cancer [9–11]. Toremifene citrate was an affinity substrate for the p-glycoprotein capable of interfering with the transport catalyzed by the p-glycoprotein [12]. Toremifene citrate in combination with paclitaxel is expected to be effective against breast cancer, however, both agents are mainly degraded via the same pathway by the hepatic enzyme cytochrome P450 [13, 14] and thier combination in treatment might induce an increase in plasma concentrations or severe side effects. We designed this prospective study to assess whether high-dose toremifene citrate in addition to paclitaxel would be safe for or beneficial to patients with metastatic breast cancer.

Patients and Methods

Patients and Eligibility criteria. Patients with metastatic breast cancer were considered for enrollment. Eligibility criteria were as follows: 1) age of 80 years or younger; 2) Eastern Cooperative Oncology Group (ECOG) performance status of 2 or less; 3) recovery from the toxic effects of previous therapy; 4) adequate bone marrow, liver and renal function; 5) without severe cardiac disease; and 6) more than 3 months predictive survival. Eligibility was independent of estrogen receptor status. Previous treatments including taxanes were not considered in the eligibility criteria. This study was performed at the Shikoku Cancer Center. The protocol was approved by the institutional review board of Shikoku Cancer Center and was carried out in accordance with the Helsinki Declaration. All patients gave their written informed consent before entry and the participants' identification codes were used for unequivocal identification of the patients. Patients were excluded if they had a high risk of a poor outcome because of concomitant nonmalignant disease, an active double cancer, and any other reason for which the investigator judged the patient to be unsuited for inclusion or unable to cooperate in the study.

Study design. Paclitaxel was administered intravenously on day 1, 8, 15, 32 and 39 and oral toremifene was administered daily from day 18. Paclitaxel was administered by intravenous infusion for 1.5 h at a dose of 80 mg/m^2 and toremifene was administered at 120 mg/body once every day (Fig. 1). This study was stopped on day 39, after which, paclitaxel was administered weekly for 3 consecutive weeks, followed by an one-week rest period and toremifene was concurrently administered orally every day. Prophylactic colony-stimulating factor (G-CSF) was used to determine whether neutropenic complications had occurred in a previous cycle.

Given the lack of appropriate pharmacological data, many questions remain about the use of toremifene for reversal of MDR including optimal dose and optimal schedule. In an *in vitro* experiment, a toremifene concentration of more than 2 μM reversed resistance, but this phenomenon was shown to be highly influenced by serum proteins *in vivo* [15]. In patients receiving toremifene to reverse doxorubicin resistance, it must be assumed that toremifene was extensively protein bound (>95%) and that toremifene concentrations in the order of >10 μM were required to overcome the effects of protein binding in plasma [15]. On the basis of pharmacological studies [16, 17], a dose of 120 mg per day was enough to maintain the plasma concentration necessary to reverse drug resistance. In addition, the time required to achieve a steady-state plasma concentration of toremifene and its metabolites was more than 2 weeks [18]. The present regimen was designed with these data in mind.

Safety evaluation. On the day before the

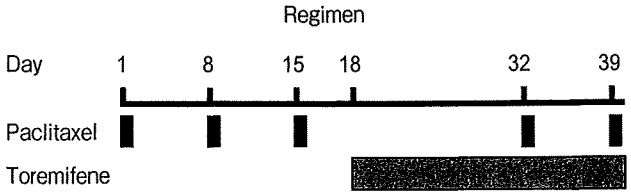

Fig. 1 Treatment schedule of weekly paclitaxel and toremifene.

administration of paclitaxel, laboratory tests were performed as follows; complete blood cell counts, differential white blood cell count, serum glutamic oxaloacetic transaminase, serum glutamic pyruvic transaminase, lactate dehydrogenase, gamma-glutamyl transpeptidase, cholinesterase, total cholesterol, electrolytes, total bilirubin, direct bilirubin, alkaline phosphatase, leucine aminopeptidase, total protein, albumin, albumin/globulin ratio, blood urea nitrogen, triglyceride, zinc sulfate turbidity test, thymol turbidity test, carcinoembryonic antigen, carbohydrate antigen 15-3, urinalysis and creatinine clearance. Doctors also interviewed patients to take a history of adverse events and physical examination. Toxicities were evaluated according to National Cancer Institute-Common Toxicity Criteria (NCI-CTC) version 2.0. The primary end point was the incidence of adverse events.

Evaluation of response. The objective response to chemotherapy was evaluated by the General Rules for Clinical and Pathological Recording of Breast Cancer (The Japanese Breast Cancer Society. 14th edition). Response assessment was performed every 1 or 2 months by serial clinical, radiographic, or computed tomographic measurement. A complete response (CR) was defined as the disappearance of all evidence of cancer for at least 4 weeks, and a partial response (PR) was defined as less than a complete response, but more than a 50% reduction of tumor volume for at least 4 weeks, without any evidence of new lesions or progression. No change (NC) was defined as less than a 50% reduction or less than a 25% increase with no new lesions. Progressive disease (PD) was defined as more than a 25% increase in a solitary lesion or the appearance of new lesions. Stable disease (SD) was defined as neither sufficient shrinkage to qualify for PR nor a sufficient increase to qualify for PD for more than 6 months. We also defined the disease control rate as the sum of CR, PR and SD to evaluate the potential benefits of this treatment.

Time to treatment failure. Time to treatment failure was calculated by the Kaplan-Meier method from the day of the initiation of the concurrent administration of toremifene and paclitaxel until the date of progression, death (any cause) or withdrawal owning to an adverse event, or patient refusal. StatView 5.0 software (SAS Institute, Inc., Cary, NC, USA) was used throughout this study.

Results

This study was carried out between December, 2003, and June, 2004, and enrolled a total of fifteen women who had metastatic breast cancer. Characteristics of patients are listed in Table 1. There were 15 women with an average age of 53.0 years. Thirteen patients had a performance status of 2 or less. Two patients had a performance status of 3, because of metastasis to vertebrae which obliged them to be bedridden however, they were considered capable of tolerating the treatment. Frequent metastatic tumor

Table 1 Patient characteristics

Total patients		15 women	
Age (range)		53.0±12.8 (33−77) yrs.	
Performance status	0	8	
	1	2	
	2	3	
	3	2	
Menopausal state	Premenopausal	6	
	Postmenopausal	9	
Prior treatment	Anthracycline	14	
	Taxane	11	(Paclitaxel: 9, Docetaxel: 9)
	5-FU	10	
	Endocrine	14	
Metastatic site	Bone	11	
	Lung	8	
	Liver	10	
	Locoregional	7	
	Others	6	

sites included the bone in 11 patients, the liver in 10 patients and the lung in 8 patients and metastases to 3 or more sites were observed in 7 patients. A total of 11 patients (73%) had received prior taxane therapy. Two patients had received paclitaxel, 2 patients (1 in a neoadjuvant setting) had received docetaxel, and 7 patients (1 who received docetaxel in a neoadjuvant setting) had received both. There was no patient who had received taxane therapy in an adjuvant setting. Characteristics of primary lesions are shown in Table 2. Twelve patients had recurrent disease; 10 of these after a curative operation and 2 patients after neoadjuvant chemotherapy and a curative operation. Three patients had metastatic disease on first arrival; 2 had received chemotherapy and surgery because their quality of life was impaired, and 1 patient received only chemotherapy. Eleven patients tested positive for estrogen receptors. No patients showed strong HER2 expression.

Table 2 Characteristics of initial tumor

Initial tumor site	Right	6
	Left	8
	Bilateral	1
Initial stage	I	1
	II	6
	III	4
	IV	3
	Unknown	1
Estrogen receptor	Positive	11
	Negative	4
HER2 (IHC)	0, 1+	13
	2+	2

A total of 112 accomplished combination treatment cycles (median 7.5, range 1–25) were administered.

Non-hematological toxicities are listed in Table 3A. There were no patients with grade 3 or greater toxicity. Frequent toxic symptoms included nausea, vomiting, alopecia, myalgia, arthralgia, and flushing. During the combination therapy, vaginal discharge was found in 3 patients. Hematological toxicities are noted in Table 3B. Only 1 patient (6.7%) had grade 3 febrile neutropenia. According to the lipid effects, hypercholesterolemia was improved but hyperglyceridemia worsened. Overall the therapy was generally well tolerated and there were no toxicity-associated deaths.

Table 4 summaries the results of chemotherapy. Of all patients, 1 partially responded and the response rate was 6.7%. Ten patients (66.7%) showed no change and 4 of them (26.7%) were stabilized for 6 months or more. The disease control rate summarizes complete responses, partial responses and stable disease, thereby accounting for the overall benefit from treatment, and was 33.3% (5 of 15 patients). Four patients (26.7%) had progressive disease. Fig. 2 shows Kaplan-Meier estimates of time to treatment failure. Median time to treatment failure was 2.7 months.

Discussion

Toremifene citrate has been shown to be an affinity substrate for the p-glycoprotein [12] and has chemosensitizing activity in MDR-positive cells at concen-

Table 3A Non-hematological toxicities

	Before entry		Paclitaxel		Paclitaxel+toremifene	
	G1	G2	G1	G2	G1	G2
Nausea/vomiting	1	0	5	0	6	1
Stomatitis	0	0	2	0	3	1
Alopecia	6	4	8	6	2	13
Sensory neuropathy (Numbness)	10	0	11	0	11	1
Myalgia/Arthralgia	2	0	3	0	5	1
Flushing	0	0	14	0	13	0
Fatigue	3	0	8	0	7	2
Taste disturbance	1	0	3	0	3	0
Edema	0	3	3	3	2	3
Lethargy	0	0	3	0	3	0
Vaginal discharge	0	0	0	0	0	3
Cough	4	0	4	0	4	0

Table 3B Hematological toxicities

	Before entry				Paclitaxel				Paclitaxel+toremifene			
	G1	2	3	4	G1	2	3	4	G1	2	3	4
Leukopenia	0	0	0	0	4	5	2	0	3	2	1	0
Hemoglobin decreased	3	1	0	0	7	2	0	0	4	4	0	0
Febrile neutropenia	0	0	0	0	0	0	0	0	0	0	1	0
Glutamic oxaloacetic transaminase increased	3	1	0	0	6	0	0	0	2	1	0	0
Glutamic pyruvic transaminase increased	3	0	0	0	7	0	0	0	3	1	0	0
Bilirubin increased	1	0	0	0	0	0	0	0	0	0	0	0
Gamma-glutamyl transpeptidase increased	3	0	1	0	4	2	0	0	0	5	0	0
Alkaline phosphatase increased	6	1	0	0	9	0	0	0	4	1	0	0
Hypoalbuminemia	2	0	0	0	4	0	0	0	6	0	0	0
Hypercholesterolemia	7	0	0	0	7	0	0	0	4	0	0	0
Hypertriglyceridemia	5	0	0	0	6	0	0	0	7	0	0	0
Proteinuria	2	0	0	0	5	0	0	0	3	0	0	0
Hematuria	3	0	0	0	4	0	0	0	2	0	0	0

Table 4 Summary of efficacy results: response rate

Tumor response		No. of patients (%)
CR		0 (0%)
PR		1 (6.7%)
NC	≧6 months	4 (26.7%)
	<6 months	6 (40%)
DCR		5 (33.3%)
PD		4 (26.7%)

CR, complete response; PR, partial response; NC, no change; DCR, disease control rate; PD, progression disease.

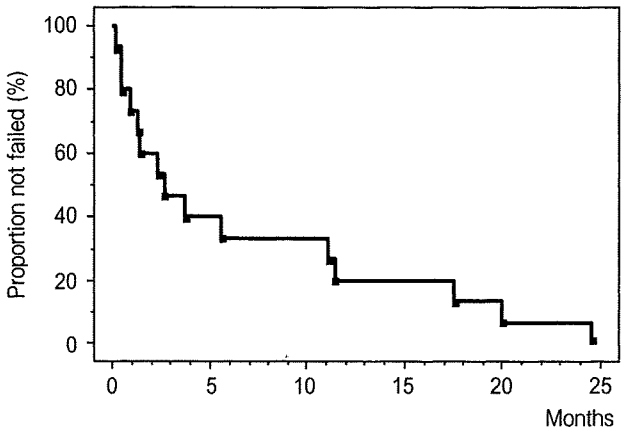

Fig. 2 Kaplan-Meier curve for time to treatment failure.

trations that are achieved in humans with minimal toxicity, although the mechanism underlying the modulation of multidrug resistance is unknown [19–22]. The development of MDR is one of the major mechanisms by which cancer becomes refractory to chemotherapeutic agents [21] and mechanisms of the MDR phenotype may involve p-glycoprotein expression, topoisomerases, and multidrug resistance-associated protein [7]. P-glycoprotein is overexpressed in approximately 40% of breast cancers and is associated with resistance to drugs of plant or bacterial origin [7]. In addition, drug resistance may arise with high baseline levels or increased expression levels of p-glycoprotein as a consequence of treatment [23]. A meta-analysis by Trock BJ *et al.* showed that patients are twice as likely to be MDR-positive following treatment, suggesting that treatment increased the expression of p-glycoprotein [7, 23].

A major problem with many reversing agents is that they can significantly alter the pharmacokinetics of the cytotoxic agents with which they are coadministered and increase the toxicity of the regimen [23, 24]. Valspodar and elacridar were developed as p-glycoprotein inhibitors in clinical trials [25]. These inhibitors modified the pharmacokinetic parameters of chemotherapeutic agents, which suggests that p-glycoprotein inhibition mediates the metabolism of anticancer drugs. However, Dofequidar fumarate, a new p-glycoprotein inhibitor, was shown to improve the progression-free survival of metastatic breast cancer patients, but it did not modify the area under the curve (AUC) of doxorubicin in a study by Saeki *et al.* [26]. Toremifene is extensively metabolized by CYP3AP and to a minor extent, by other hepatic