breast or ovarian cancer including nulliparity, early menarche, advanced age, obesity, the use of hormone replacement therapy, and lifestyle characteristics other than family history. These factors were not included in the self-administered questionnaire used in this study. Although family history is a major risk factor for both breast and ovarian cancer, some selection bias may thus have influenced the results. The second limitation is the choice of referents. We used hospital-based patients as controls. It is sometimes pointed out that there are discrepant characteristics between the general population and hospital-based references. Such potential limitation should be considered before drawing definitive conclusions from the current study. The third criticism is the accuracy of self-reported family history data. It is important to determine how reliable these data are to avoid any possible recall bias. A number of studies have found that women can provide accurate (>90% accuracy) and reliable information about their family history of cancer.[17,18] It is reported if there was any recall bias operating in the reporting of family histories, the effect of the risk estimates would thus have been negligible.[19]

The hereditary breast/ovarian cancer syndrome is thought to be largely attributable to mutations in the BRCA1 and BRCA2 genes. Molecular genetic testing for BRCA1 and BRCA2 cancer-predisposing mutations is available on a clinical basis for probands who are identified to be at high risk for having a mutation of those genes. Approximately 7% of the patients with breast cancer and 10% of the patients with ovarian cancer are estimated to have a mutation in those cancer susceptibility genes.[20] Women who carry a deleterious BRCA1 or BRCA2 mutation have a 50–80% lifetime risk of breast cancer and a 10–40% lifetime risk of ovarian cancer.[21] The recognition of a BRCA mutation is often valuable in the decision making of patients with newly diagnosed breast or ovarian cancer. Once a mutation has been identified in the proband, genetic counseling and testing can be offered to unaffected family members. A systematic approach to collecting family histories and the use of risk models for mutation of those genes are required to identify those patients who need genetic testing.

Using several currently available models, from 3.3 to 6.0% of female patients without a history of breast or ovarian cancer have been shown to have family histories suggestive of a mutation and are therefore eligible for additional evaluation.[6,22]

Among the patients with a personal history of breast and/or ovarian cancer, the proportion with significant family histories may be much higher. Shannon *et al.*[23] reported a rate of 22% of high-risk patients among a cohort of 50 women with a history of breast or ovarian cancer, as determined by a genetic counselor. This incidence is highest among the patients with an Ashkenazi ancestry (47.3%) and among those with a personal history of ovarian cancer (35.9%).

Probability models have been developed to estimate the likelihood that an individual family has a mutation in BRCA1 or BRCA2. Among those models the Myriad mutation prevalence tables and the BRCAPRO model are the most widely used.

Dominguez *et al.*[24] reported that 20.6% of patients with a personal history of breast or ovarian cancer had a family history suggestive of a 10% risk of a BRCA1 or BRCA2 genetic mutation according to Myriad tables. In this study only 7.5% had a 10% risk of carrying a BRCA1 or BRCA2 mutation using the same model. Consistent with this result, the frequency of the BRCA1 mutation has been reported to be much lower in Japanese breast/ovarian cancer families.[25] The incidence rate of breast and ovarian cancer in Japan (74.4 per 100 000 women) is much lower than that in USA (120.2). The difference in the incidence rate may be partially due to the lower incidence of the BRCA mutation in Japan. Both BRCA1 and BRCA2 mutations have been reported to actually be more prevalent among women with ovarian cancer. The prevalence of BRCA mutations in ovarian cancer patients is reported to be approximately 12%[26] whereas that in breast cancer patients is approximately 5%.[20] Dominiquez *et al.*[24] reported that 16.9% of women (non-Ashkenazi) with breast cancer versus 30.9% of those with ovarian cancer were considered to have a 10% risk for carrying a mutation. The risk for carrying a mutation according to cancer type did not show a difference in the current study: 7.0% for breast cancer and 8.7% for ovarian cancer. It has been suggested that genetic testing should be considered for women who have been diagnosed with invasive ovarian cancer regardless of the family history, due to the high incidence of BRCA mutations.[27] The current results indicate that the incidence of a high-risk family history in Japanese ovarian cancer patients may be much lower due to the difference in genetic background. Various models are currently applied to evaluate the patient's risk and to assist in the decision of whether to recommend testing, however, ample discrepancies exist between them and the risk probabilities they generate. The Myriad model is a reasonable model to use as a first screening of high-risk women, because it is simple and fast.

However, Asian women may not be accurately represented by this method because they represent only 1.1% of the individuals previously analyzed by Myriad laboratories. Moreover, the number of patients was relatively small in the current study, especially because the number of patients with ovarian cancer was only 289. A larger scale case–control study or cohort studies are required to confirm these results.

In conclusion, the incidence of a family history of breast and ovarian cancer and the prevalence of hereditary breast/ovarian cancer risk was assessed in Japanese women. The self-administered questionnaire is a simple, fast and effective method for detecting high-risk patients based on their family history. Obtaining a detailed breast and ovarian cancer family history and application of the Myriad model are useful for identifying women at elevated genetic risk of breast/ovarian cancer. Therefore, estimating the prevalence of hereditary breast/ovarian cancer syndrome is considered to have significant implications for patient management, as well as for the capacity of risk assessment and testing.

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ORIGINAL ARTICLE

Pharmacokinetic analysis of a combined chemoendocrine treatment with paclitaxel and toremifene for metastatic breast cancer

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Abstract
Background Multidrug resistance protein could be a target for improving the efficacy of paclitaxel (PXL). Toremifene (TOR) may moderate P-gp-related drug resistance in vitro. Some P-gp moderators may change the pharmacokinetic parameters of PXL in vivo. A pharmacokinetic (PK) study in metastatic breast cancer patients (MBC) was conducted to determine the safety and efficacy of PXL and TOR.

Method and patients Fifteen patients received 80 mg/m^2 PXL (i.v.) weekly and 120 mg/body TOR (p.o.) daily. For the pharmacokinetic study, PXL was administered on days 1, 8, 15, 32, and 39; TOR was given from day 18 to the end of study. On days 1, 8, 15, 18, 32, and 39, blood samples were collected from the patients who received either PXL alone or PXL + TOR, and these were analyzed by high-performance liquid chromatography.
Results Among the 15 patients enrolled in the study, one showed a partial response, and eight had a stable disease. TOR caused no specific adverse events that were greater than grade 3, and its toxicity profile in combination with PXL was similar to that of PXL monotherapy. The PK profile of PXL was similar with or without TOR. The PK parameters of PXL indicated no inter- or intra-patient variability in previously treated patients with MBC. No increased PXL toxicity was observed.
Conclusion The PK profile of combined PXL and TOR was similar to that of PXL monotherapy. The addition of TOR to PXL in previously treated patients with MBC appears safe.

Keywords Breast cancer · Chemoendocrine therapy · Drug resistance · Antiestrogens · Toremifene · Paclitaxel

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Introduction

ATP-binding cassettes (ABC) may play an important role in chemotherapy, because some malignant tumors have a congenital resistance to anticancer agents, which can be substrates of either P-glycoprotein (P-gp) or multidrug resistant protein 2 (MRP2) [1–7]. Chemotherapy may improve the survival rate of breast cancer patients, and

endocrine treatment may also be clinically beneficial [8–13]. Antiestrogens, tamoxifen, and toremifene (TOR) were shown to be effective in hormone-receptor-positive breast cancer patients [14]. For hormone-receptor-negative breast cancer, chemotherapy significantly improved the overall survival rate in primary breast cancer patients and progression-free survival in metastatic breast cancer patients [15–17]. Recently, a new category for endocrine responsiveness, "endocrine response uncertain," was identified in primary systemic treatment [18]. Chemoendocrine therapy is recommended because sensitivity to chemotherapy alone is relatively poor in breast cancer patients with hormone-receptor-positive breast cancer [12, 19]. However, the timing of chemotherapy combined with tamoxifen has been discussed [20]. The role of P-gp has been investigated in relation to hormone receptor status and drug-resistance, and P-gp may be involved in either endocrine response or chemosensitivity [19]. P-gp is considered to be one of the factors that predicts the success of chemotherapy; therefore, this protein remains a target in efforts to improve treatment failure in patients with advanced and recurrent breast cancer. To overcome drug resistance, P-gp modifiers will be needed for optimal chemotherapeutic results.

Antiestrogens may moderate P-gp-related drug resistance in vitro [21–23]. A synergistic effect of PXL and antiestrogens was observed in a multidrug-resistant cell line [24]. This synergistic effect was more potent when PXL was combined with TOR than with tamoxifen. In addition, TOR had a more synergistic effect than tamoxifen when used in combination on the proliferation of doxorubicin-resistant MCF-7 cells [25]. PXL was not effective when a P-gp gene was transfected into breast cancer cells [26]. Efflux of PXL from cancer cells might be mediated by P-gp, and the sensitivity of PXL might be mainly dependent on the expression of P-gp in breast cancer [7, 27, 28]. P-gp inhibitors may improve the sensitivity of PXL [29]. In this regard, antiestrogens may modify P-gp function and are potential candidates for P-gp inhibitors. However, some P-gp inhibitors such as valspodar and elacridar alter the pharmacokinetic parameters of anticancer agents, because these inhibitors moderate the function of P-gp in normal epithelial cells of renal microtubules or canalicular membrane of hepatocytes [30]. In addition, drug–drug interactions between PXL and TOR may affect the pharmacokinetic parameters of those two drugs, since both agents can be metabolized with CYP3A4 [31, 32]. To determine the pharmacokinetic parameters resulting from treatment with a combination of PXL and TOR, we conducted a pharmacokinetic study in metastatic breast cancer patients.

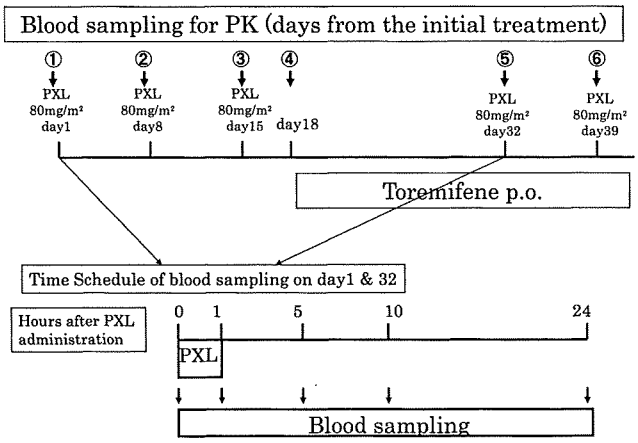

Fig. 1 Treatment and blood sampling schedule. Blood samples were collected on days 1, 8, 15, 18, 32, and 39. For the pharmacokinetic study, patients received paclitaxel monotherapy on days 1, 8, 15, 32, and 39. Patients were given toremifene (120 mg) orally from day 18 to the end of study. Blood collection was performed on days 1, 8, and 15 for the PK analysis of PXL. On day 18, blood sampling was performed before and after TOR administration for the PXL and TOR PK analyses. On days 32 and 39, we collected blood samples for the PK analyses of PXL and TOR

Material and methods

Patient eligibility criteria

Patients with histologically confirmed metastatic breast cancer, with a Eastern Clinical Oncology Group (ECOG) performance status of 0–2, 40–70 years of age, and with adequate liver and renal function, were eligible. In addition, patients who are planning to receive paclitaxel in practice and those who provided written informed consent were considered for the study.

Patient exclusion criteria

Patients were excluded if they had severe complications or were taking drugs known to be metabolized by CYP3A4, such as phenylalanine, phenobarbital, rifampicin, and carbamazepine.

Treatment

PXL formulated in Cremophor EL and dehydrated alcohol (1:1, v/v, 6 mg/mL, and taxol) was administered (i.v.). PXL (80 mg/m^2) was given for 1 h on days 1, 8, and 15 in a cycle. A cycle consisted of one week of treatment followed by one week off. Toremifene (TOR) (120 mg/(body day^{-1})) was administered orally from day 1 to day 21. Patients were repeatedly treated with a combination of PXL and TOR as long as disease

Table 1 Patient characteristics in 15 patients

Age (year)	53.0 ± 12.8 (range 33–77)
Performance status	
0–2	11
3	2
Menopausal state	
Premenopausal	6
Postmenopausal	9
Prior treatment	
Anthracycline	14
Taxane	11 (paclitaxel 9, docetaxel 9)
5FU	10
Endocrine	14
Metastatic site	
Bone	11
Lung	8
Liver	10
Others	13
ER	
Positive	11
Negative	4
Her2	
Positive	0
Negative	15

progression or unmanageable severe adverse events were defined.

For the pharmacokinetic study, PXL was initially administrated alone on days 1, 8, and 15. Beginning on day 18, TOR (120 mg) was given daily. On day 22, PXL administration in the first cycle was skipped. Blood samples were collected on day 32.

Blood sampling

Blood samples for pharmacokinetic (PK) analysis were collected on days 1, 8, 15, 18, 32, and 39 (Fig. 1). On day 1, blood samples were collected at six different time points for PK analysis of PXL alone: (1) before PXL administration, (2) 1 h after PXL administration, (3) at the end of PXL administration, (4) 3.5 h after the end of PXL administration, (5) 8.5 h after the end of PXL administration, and (6) 22.5 h after the end of PXL administration. On days 8 and 15, blood was collected 1 h after the end of PXL infusion. On day 18, blood samples were collected pre- and 1 h after TOR administration. Patients received 120 mg TOR at 8 a.m. after a meal. On day 32, blood samples from the patients who received 120 mg TOR p.o. at 8 a.m. were collected at six different time points for PK analysis of PXL, TOR and *N*-demethyltoremifene (TOR-1): (1) before PXL

Table 2 Pharmacokinetic parameters for paclitaxel (dose: paclitaxel 80 mg/m^2) without toremifene

Patient no.	$T_{1/2}$ Z (h)	AUC last (μg h/mL)	AUC inf. (μg h/mL)	CL tot [L/(h m^{-2})]	MRT (h)	V_{ss} (L/m^2)
1	9.4007	11.0846	11.4515	6.986	2.9095	20.3258
2	8.5093	6.0958	6.354	12.5906	3.6676	46.1774
3	6.9109	8.1346	8.3909	9.5341	3.384	32.2634
4	8.5592	8.1326	8.4103	9.5121	3.1104	29.5868
5	10.5148	5.9439	6.3298	12.6386	4.6365	58.5981
6	7.9377	6.594	6.8432	11.6905	3.4779	40.6582
7	10.0314	12.2528	12.9084	6.1975	4.0461	25.0759
8	8.9157	8.7407	9.1737	8.7206	4.0759	35.5442
9	8.6034	9.4411	9.8746	8.1016	3.8592	31.2651
10	10.0948	7.4109	7.983	10.0213	5.4547	54.663
11	8.4894	14.1294	14.7499	5.4238	3.7968	20.5932
12	8.3235	7.9264	8.1943	9.7629	3.1317	30.5742
13	7.9475	11.289	11.6655	6.8578	3.1531	21.6234
14	10.306	8.1792	8.562	9.3436	3.6288	33.9064
15	9.5512	5.7761	6.1572	12.9929	4.9199	63.9236
Mean	8.9	8.74	9.14	9.36	3.8	36.3
SD	1	2.47	2.56	2.39	0.7	13.8
Median	8.6	8.13	8.41	9.51	3.7	32.3
Maximum	10.5	14.13	14.75	12.99	5.5	63.9
Minimum	6.9	5.78	6.16	5.42	2.9	20.3

Paclitaxel 80 mg/m^2 was administrated intravenously for 1 h on day 1

The following pharmacokinetic parameters were evaluated: $T^{1/2}$ *Z* half-life in the terminal phase, *AUC last* area under the concentration–time curve up to the last measurement time point, *AUC inf.* area under the concentration–time curve up to infinite time, *MRT* mean residence time, *CL tot* total body clearance, V_{ss} volume of distribution at steady state

administration; (2) 1 h after PXL administration; (3) at the end of PXL administration; (4) 3.5 h after the end of PXL administration; (5) 8.5 h after the end of PXL administration; and (6) 22.5 h after the end of PXL administration. On day 39, a sample was collected 24 h after PXL infusion and 120 mg TOR p.o. administration for the PK analysis of PXL and TOR.

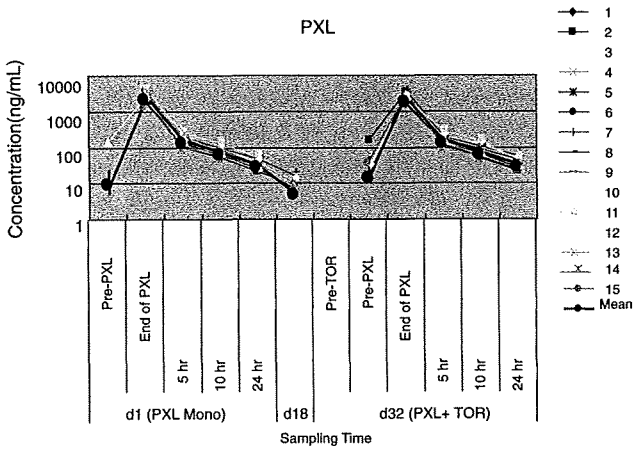

Fig. 2 Pharmacokinetic profile for paclitaxel in 15 patients. On day 1, the concentration of PXL without TOR at each sampling time is shown on the *left side* of this figure. The PXL pharmacokinetic profiles on day 32 with TOR are drawn on the *right side*

For the pharmacokinetic analysis of TOR and TOR-1, blood samples were collected in the morning after oral intake of 120 mg TOR. Samples were centrifuged at 2,100×*g* for 10 min at 4 °C, and the plasma fraction was collected and stored at −20 °C until analysis. PXL concentrations in plasma samples obtained from a peripheral vein were measured using a liquid chromatography–mass spectrometry or mass spectrometry assay.

Pharmacokinetic parameters

Concentrations of PXL, TOR, and TOR-1 were analyzed by high-performance liquid chromatography (HPLC). PK parameters were calculated using WinNonlin Professional software (v.5.0.1; Pharsight Corporation, Mountain View, CA, USA). Noncompartmental analysis was performed. Statistical analysis was performed using the *t* test.

Results

Patient background

Fifteen patients with metastatic breast cancer were enrolled. Fourteen patients received anthracycline, and 11 were treated with PXL and docetaxel (Table 1). In addition, 14 patients had previously received endocrine treatment.

Table 3 Pharmacokinetic parameters for paclitaxel (dose: paclitaxel 80 mg/m^2) with toremifene 120 mg/body

Patient no.	$T_{1/2}$ Z (h)	AUC last (μg h/mL)	AUC inf. (μg h/mL)	CL tot [L/(h m^{-2})]	MRT (h)	V_{ss} (L/m^2)
1	8.8038	5.3892	5.7266	13.9698	4.8959	68.395
2	8.6899	9.7528	10.0182	7.9854	2.6128	20.8646
3	8.0997	8.8134	9.1517	8.7416	3.5169	30.743
4	10.0986	7.7933	8.2006	9.7554	3.9509	38.5432
5	8.0958	8.2835	8.5647	9.3406	3.2274	30.1462
6	8.1442	5.8725	6.1207	13.0704	3.7166	48.578
7	11.1207	12.0039	12.8667	6.2176	4.911	30.5343
8	11.1569	6.45	7.1505	11.1881	6.8686	76.8468
9	8.0073	6.4636	6.7921	11.7783	4.3609	51.3635
10	9.7683	7.5252	8.2268	9.7243	6.4098	62.3314
11	7.3607	8.9564	9.4219	8.4909	4.861	41.2741
12	8.6712	7.8764	8.1776	9.7829	3.3407	32.6813
13	9.2115	8.2912	8.6769	9.2199	3.8067	35.0977
14	9.8566	7.8927	8.2434	9.7048	3.535	34.3065
15	8.6802	8.4938	8.7807	9.1109	3.0571	27.8527
Mean	9.1	7.99	8.41	9.87	4.2	42
SD	1.1	1.64	1.71	1.96	1.2	16.3
Median	8.7	7.89	8.24	9.7	3.8	35.1
Maximum	11.2	12	12.87	13.97	6.9	76.8
Minimum	7.4	5.39	5.73	6.22	2.6	20.9
Paired *t* test	NS	NS	NS	NS	NS	NS

On day 32, 15 patients received paclitaxel (80 mg/m^2) + toremifene (120 mg)

The following pharmacokinetic parameters were evaluated: $T_{1/2}$ Z half-life in the terminal phase, *AUC last* area under the concentration–time curve up to the last measurement time point, *AUC inf.* area under the concentration-time curve up to infinite time, *CL tot* total body clearance, V_{ss} volume of distribution at steady state. Each parameter was statistically analyzed between paclitaxel alone and paclitaxel + toremifene. *NS* $P > 0.05$ compared with paclitaxel alone

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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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

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

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											

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

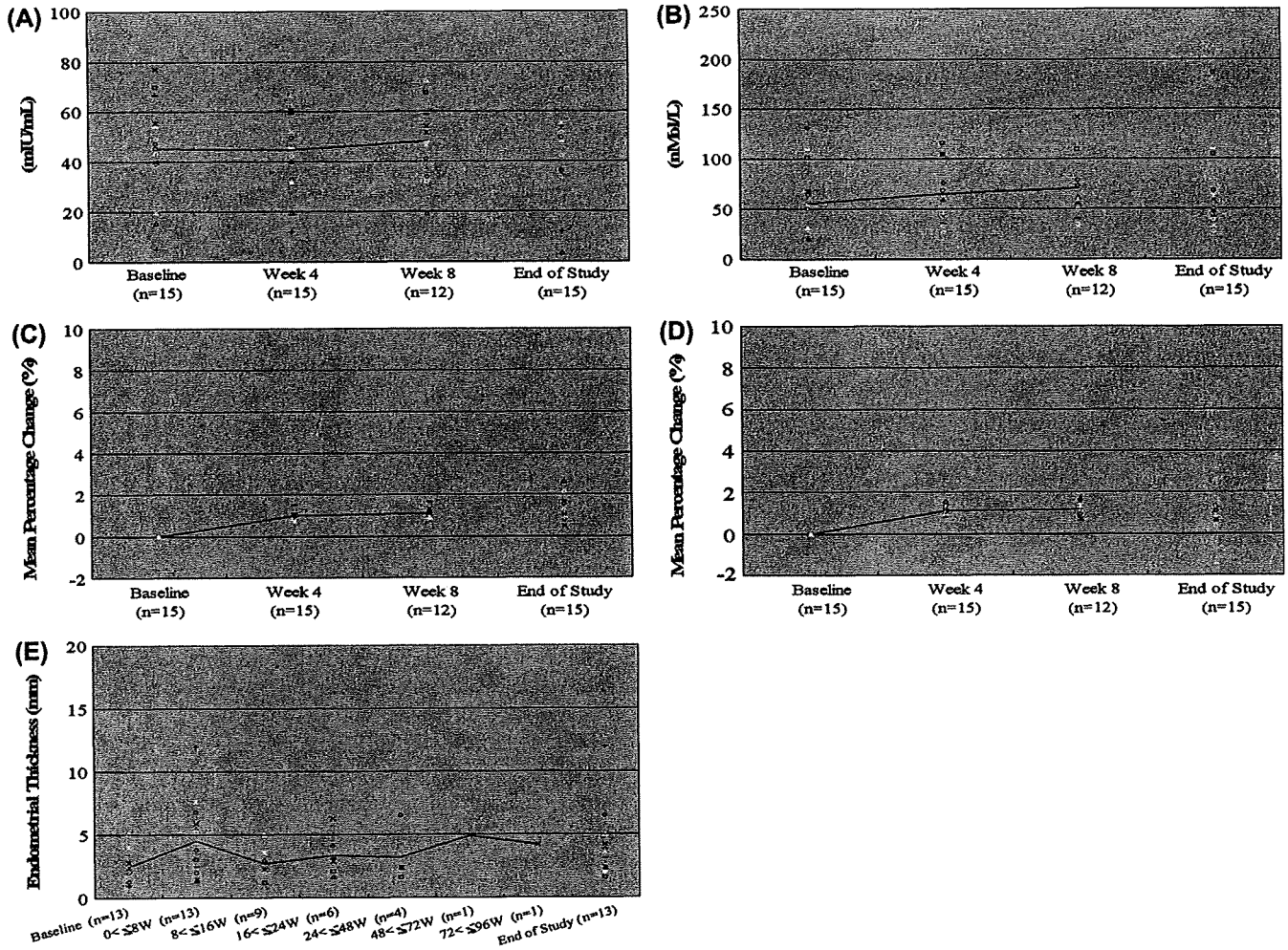

Figure 1. Individual and mean plasma concentration-period profiles [(A) FSH and (B) SHBG], relative mean changes-period profiles [(C) I CTP and (D) osteocalcin] and endometrial thickness -period profiles (E) of all patients.

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

Drug and metabolite	Dose (mg)	Concentration (ng/ml)					
		Pre-dose	2 weeks	4 weeks	6 weeks	8 weeks	12 weeks
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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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.

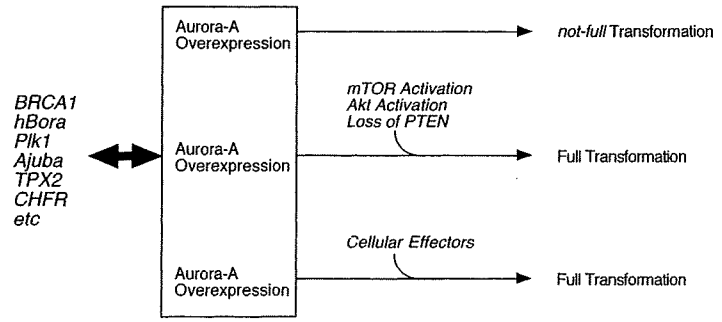

Figure 1. Model of Aurora-A cell transformation. Physiological regulation of Aurora-A kinase activity is by BRCA1, hBora, Ajuba, TPX2 an dPlk1 etc, however, cell transformation by Aurora-A requires additional oncogenic events, such as constitutive activation of mTOR/Akt pathway and loss of PTEN tumor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

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Conflict of Interest

The authors have declared that no conflict of interest exists.

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