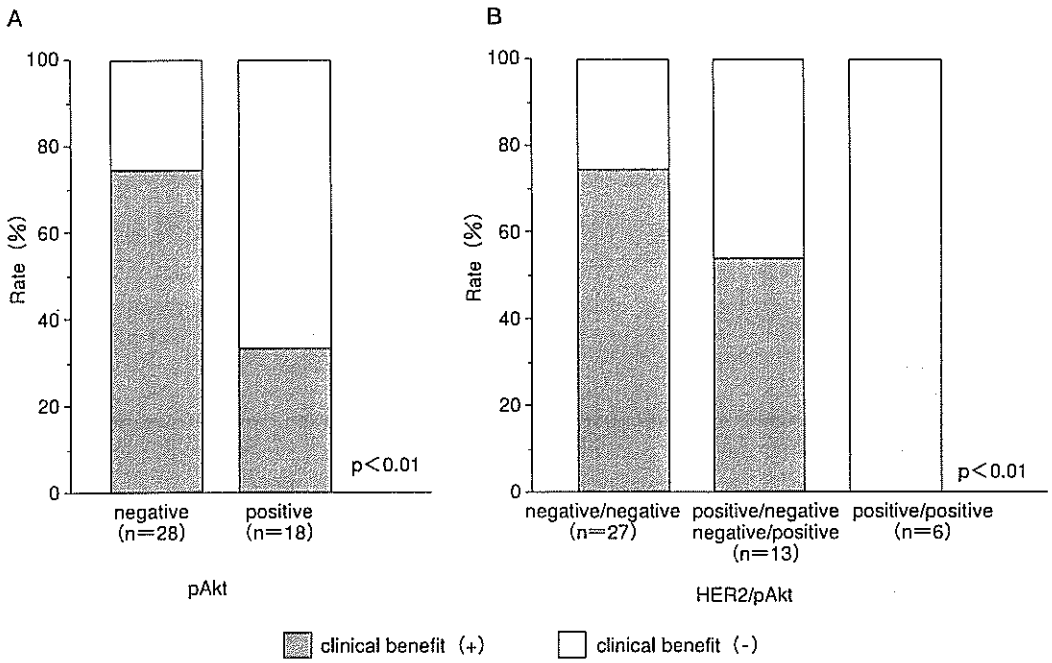

Fig 3. Relationship between clinical benefit of hormone therapy and pAkt expression (A) or pAkt/HER2 expression (B)
A. In the pAkt-positive patients, endocrine therapy had significantly worse efficacy than in pAkt-negative patients (p<0.01). B. The clinical benefit was the smallest in patients positive for both HER2 and pAkt (p<0.01).

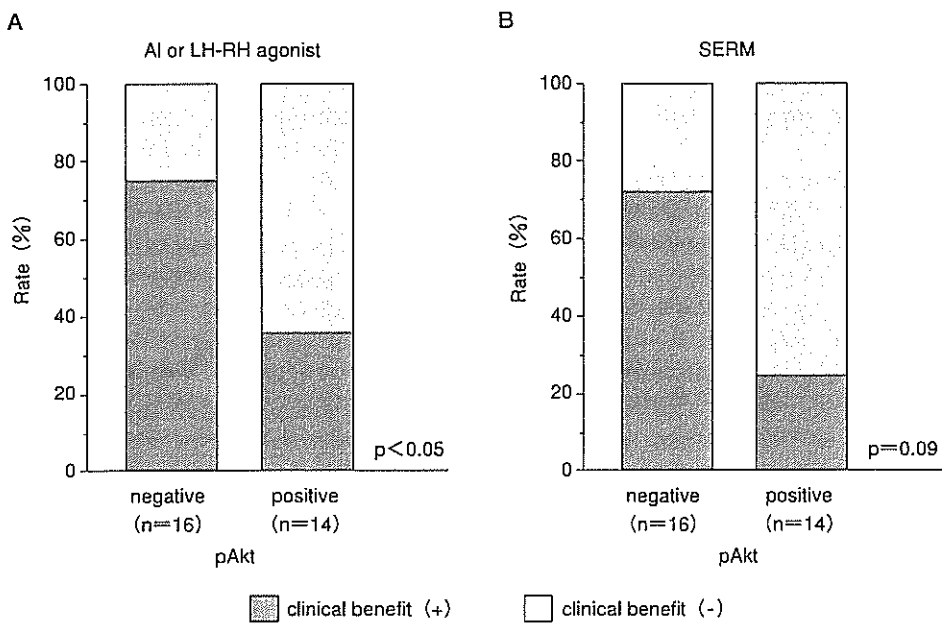

Fig 4. Relationship between the clinical benefits and the status of pAkt by endocrine agent; estrogen depletion therapy, such as aromatase inhibitors or LH-RH agonist (A), SERM (B)
A. In pAkt-positive patients, the clinical benefit rate of estrogen deprivation therapy was significantly lower than that in pAkt-negative patients (p<0.05). B. The clinical benefit of SERM tended to be smaller in the pAkt-positive patients (p=0.09).

Discussion

Akt/PKB is a serine/threonine kinase, which is a downstream effector of PI3-K. The major functions of the PI3K/Akt signal pathway are to promote growth-factor-mediated cell growth, proliferation, migration and survival[9]. Because the activation of the PI3K/Akt pathway induces resistance to the apoptotic response, the inhibition of this pathway is now considered to be a promising strategy to improve the effect of therapies for various kinds of cancers[21].

In the present study, we found that pAkt expression correlated significantly with HER2 overexpression. This finding was consistent with previous reports[13, 22]. In addition, we found that LOH of the PTEN locus is significantly associated with Akt activation. PTEN LOH may diminish PTEN function and therefore induce Akt activation. Interestingly, the expression of pAkt was more significantly associated with coexistence of PTEN LOH and HER2 overexpression, both of which induce Akt activation. On the other hand, an inverse correlation was found between pAkt and PR expression ($p<0.05$). A recent study demonstrated that expression of the progesterone receptor was reduced via the PI3K/Akt pathway[23], and this finding may support our results.

We then investigated the association between Akt activation and the efficacy of endocrine therapy in metastatic breast cancer. Recent studies suggest high Akt activity in breast carcinoma to be associated with a poor prognosis in patients with adjuvant endocrine therapy[12, 13]. However, the relationship between Akt activation and the efficacy of endocrine therapy for metastatic breast cancer has not been reported.

In the present study, we analyzed 36 cases of metastatic breast cancer who had been treated with 46 endocrine therapies. In terms of the relationship between Akt activation and the efficacy of endocrine therapy, the clinical benefit rate was significantly lower in the pAkt-positive patients ($p<0.01$). In addition, the clinical benefit was the smallest in the both HER2 and pAkt positive patients ($p<0.01$).

We thereafter investigated whether the association between pAkt and resistance to endocrine therapy differed by type of endocrine therapy. The activation of Akt has been reported to be associated with resistance to antiestrogens such as tamoxifen, however, the relationship between Akt activation and the effect of estrogen depletion therapy, such as aromatase inhibitors (AIs) or LH-RH agonist, has not yet been elucidated. Interestingly, pAkt-positivity significantly correlated with lack of efficacy of estrogen depletion therapy ($p<0.05$). In experimental studies, a constitutively active Akt mutant mimics the effect of estrogen in the absence of the ligand of the estrogen receptor[24]. These results suggest that breast cancer cells with activated Akt can survive under estrogen suppression by either aromatase inhibitors or LH-RH agonist.

In this study, we demonstrated that Akt activation was significantly associated with a poor response to endocrine therapy for metastatic breast cancer. The results of this study suggest that inhibition of the Akt signaling pathway may improve the efficacy of endocrine therapy for metastatic breast cancer. In fact, there are some either currently ongoing or planned phase II/III clinical trials of endocrine therapy, either with or without signal transduction inhibitors for locally advanced or metastatic breast cancer[6]. In combination with endocrine therapy, monoclonal antibodies such as trastuzumab, tyrosine kinase inhibitors such as gefinitib or lapanitib, and the mTOR inhibitors, CCI-779 or RAD001 were recruited in these trials. The data obtained from these studies will hopefully lead to an improvement in the treatment for breast cancer patients.

In conclusion, this study suggests that pAkt may be a useful predictor of resistance to endocrine therapy for breast cancer, while also suggesting that the inhibition of Akt may increase the efficacy of endocrine therapy.

Acknowledgements

We are grateful to Ms. Y. Kubota, Ms. N. Nakamura, Ms. T. Shishino and Ms. K. Yamashita for their valuable technical assistance. This study was supported by grants from the Ministry of Education, Culture, Sports Science, and Technology of Japan.

References

1) Osborne CK: Tamoxifen in the treatment of breast cancer. N Engl J Med, 339:1609-1618, 1998
2) Janicke F: Are all aromatase inhibitors the same? A review of the current evidence. Breast, 13:S10-18, 2004

3) Mouridsen HT, Rose C, Brodie AH, Smith IE: Challenges in the endocrine management of breast cancer. Breast, 12:S2-19, 2003
4) Lapidus RG, Nass SJ, Davidson NE: The loss of estrogen and progesterone receptor gene expression in human breast cancer. J Mammary Gland Biol Neoplasia, 3:85-94, 1998
5) Ali S, Coombes RC: Endocrine-responsive breast cancer and strategies for combating resistance. Nat Rev Cancer, 2:101-112, 2002
6) Johnston SR: Combinations of endocrine and biological agents: present status of therapeutic and presurgical investigations. Clin Cancer Res, 11:889s-899s, 2005
7) Kurokawa H, Arteaga CL: ErbB (HER) receptors can abrogate antiestrogen action in human breast cancer by multiple signaling mechanisms. Clin Cancer Res, 9:511S-515S, 2003
8) Schiff R, Massarweh SA, Shou J, Bharwani L, Mohsin SK, Osborne CK: Cross-talk between estrogen receptor and growth factor pathways as a molecular target for overcoming endocrine resistance. Clin Cancer Res, 10:331S-336S, 2004
9) Nicholson KM, Anderson NG: The protein kinase B/Akt signalling pathway in human malignancy. Cell Signal, 14:381-395, 2002
10) Stambolic V, Suzuki A, de la Pompa JL, Brothers GM, Mirtsos C, Sasaki T, Ruland J, Penninger JM, Siderovski DP, Mak TW: Negative regulation of PKB/Akt-dependent cell survival by the tumor suppressor PTEN. Cell, 95:29-39, 1998
11) Clark AS, West K, Streicher S, Dennis PA: Constitutive and inducible Akt activity promotes resistance to chemotherapy, trastuzumab, or tamoxifen in breast cancer cells. Mol Cancer Ther, 1:707-717, 2002
12) Perez-Tenorio G, Stal O: Activation of AKT/PKB in breast cancer predicts a worse outcome among endocrine treated patients. Br J Cancer, 86:540-545, 2002
13) Stal O, Perez-Tenorio G, Akerberg L, Olsson B, Nordenskjold B, Skoog L, Rutqvist LE: Akt kinases in breast cancer and the results of adjuvant therapy. Breast Cancer Res, 5:R37-44, 2003
14) Yu K, Toral-Barza L, Discafani C, Zhang WG, Skotnicki J, Frost P, Gibbons JJ: mTOR, a novel target in breast cancer: the effect of CCI-779, an mTOR inhibitor, in preclinical models of breast cancer. Endocr Relat Cancer, 8:249-258, 2001
15) Japanese Breast Cancer Society: General rules for clinical and pathological recording of breast cancer, 14 th edn. Tokyo: Kanehara; 2001.
16) Tokunaga E, Oki E, Oda S, Kataoka A, Kitamura K, Ohno S, Maehara Y, Sugimachi K: Frequency of microsatellite instability in breast cancer determined by high-resolution fluorescent microsatellite analysis. Oncology, 59:44-49, 2000
17) Ellis MJ, Coop A, Singh B, Mauriac L, Llombert-Cussac A, Janicke F, Miller WR, Evans DB, Dugan M, Brady C, Quebe-Fehling E, Borgs M: Letrozole is more effective neoadjuvant endocrine therapy than tamoxifen for ErbB-1- and/or ErbB-2-positive, estrogen receptor-positive primary breast cancer: evidence from a phase III randomized trial. J Clin Oncol, 19: 3808-3816, 2001
18) Jacobs TW, Gown AM, Yaziji H, Barnes MJ, Schnitt SJ: Specificity of HercepTest in determining HER-2/neu status of breast cancers using the United States Food and Drug Administration-approved scoring system. J Clin Oncol, 17:1983-1987, 1999
19) Seidman AD, Fornier MN, Esteva FJ, Tan L, Kaptain S, Bach A, Panageas KS, Arroyo C, Valero V, Currie V, Gilewski T, Theodoulou M, Moynahan ME, Moasser M, Sklarin N, Dickler M, D'Andrea G, Cristofanilli M, Rivera E, Hortobagyi GN, Norton L, Hudis CA: Weekly trastuzumab and paclitaxel therapy for metastatic breast cancer with analysis of efficacy by HER2 immunophenotype and gene amplification. J Clin Oncol, 19:2587-2595, 2001
20) Oki E, Baba H, Tokunaga E, Nakamura T, Ueda N, Futatsugi M, Mashino K, Yamamoto M, Ikebe M, Kakeji Y, Maehara Y: Akt phosphorylation associates with LOH of PTEN and leads to chemoresistance for gastric cancer. Int J Cancer, 117:376-380, 2005
21) Thompson JE, Thompson CB: Putting the rap on Akt. J Clin Oncol, 22:4217-4226, 2004
22) Zhou X, Tan M, Stone Hawthorne V, Klos KS, Lan KH, Yang Y, Yang W, Smith TL, Shi D, Yu D: Activation of the Akt/mammalian target of rapamycin/4E-BP1 pathway by ErbB2 overexpression predicts tumor progression in breast cancers. Clin Cancer Res, 10:6779-6788, 2004
23) Cui X, Zhang P, Deng W, Oesterreich S, Lu Y, Mills GB, Lee AV: Insulin-like growth factor-I inhibits progesterone receptor expression in breast cancer cells via the phosphatidylinositol 3-kinase/Akt/mammalian target of rapamycin pathway: progesterone receptor as a potential indicator of growth factor activity in breast cancer. Mol Endocrinol, 17:575-588, 2003
24) Stoica GE, Franke TF, Moroni M, Mueller S, Morgan E, Iann MC, Winder AD, Reiter R, Wellstein A, Martin MB, Stoica A: Effect of estradiol on estrogen receptor-alpha gene expression and activity can be modulated by the ErbB2/PI 3-K/Akt pathway. Oncogene, 22:7998-8011, 2003

EUROPEAN JOURNAL OF CANCER 42 (2006) 629–635

available at www.sciencedirect.com

SCIENCE DIRECT®

journal homepage: www.ejconline.com

The association between Akt activation and resistance to hormone therapy in metastatic breast cancer

Eriko Tokunaga[a,*], *Akemi Kataoka*[b], *Yasue Kimura*[a], *Eiji Oki*[a], *Kojiro Mashino*[a], *Kojiro Nishida*[a], *Tadashi Koga*[a], *Masaru Morita*[a], *Yoshihiro Kakeji*[a], *Hideo Baba*[c], *Shinji Ohno*[b], *Yoshihiko Maehara*[a]

[a]*Department of Surgery and Science, Graduate School of Medical Sciences, Kyushu University, Maidashi 3-1-1, Higashi-ku, Fukuoka 812-8582, Japan*

[b]*Department of Breast Oncology, National Kyushu Cancer Center, Japan*

[c]*Department of Gastroenterological Surgery, Postgraduate School of Medicine, Kumamoto University, Japan*

ARTICLE INFO

Article history:
Received 17 September 2005
Received in revised form
17 November 2005
Accepted 21 November 2005
Available online 7 February 2006

Keywords:
Akt
Phosphorylated Akt
HER2
Metastatic breast cancer
Hormone therapy

ABSTRACT

In this retrospective study, the relationship between Akt activation and the efficacy of endocrine therapy for metastatic breast cancer was investigated. Thirty-six metastatic breast cancer patients, treated with endocrine therapy, were evaluated for the activation of Akt by an immunohistochemical assessment of the expression of phosphorylated Akt at Ser 473 (pAkt). The relationship between the efficacy of endocrine therapy and Akt activation, HER2 status and hormone receptor expression was also investigated. Of these 36 cases, 12 cases (33.4%) were considered to show a positive pAkt expression. In the pAkt-positive patients, endocrine therapy demonstrated a worse efficacy than in pAkt-negative patients ($P < 0.01$). pAkt positivity was also associated with a poorer objective response ($P < 0.05$). The clinical benefit rate was lower in HER2 positive groups than in HER2 negative group ($P < 0.05$). In addition, the clinical benefit was the smallest in both the HER2 and pAkt-positive patients ($P < 0.01$). Regarding the endocrine agents, the clinical benefit of estrogen deprivation therapy with aromatase inhibitor or luteinising hormone-releasing hormone agosists was significantly lower in the pAkt-positive patients than that in the pAkt-negative ones ($P < 0.05$). In addition, there was a tendency for clinical benefit of selective estrogen receptor modulator to be smaller in the pAkt-positive patients ($P = 0.09$). These findings, therefore, suggest that Akt activation induces endocrine resistance in metastatic breast cancer, irrespective of the kind of endocrine agents that were administered. Our findings suggest that the activation of Akt in the downstream pathway of HER2 plays an important role in the resistance to endocrine therapy for breast cancer. Although our study was small in scope and retrospective in design, our findings suggest that pAkt may be a useful predictor of resistance to endocrine therapy for breast cancer, while also suggesting that the inhibition of Akt may increase the efficacy of endocrine therapy.

* *Corresponding author:* Tel.: +81 92 642 5466; fax: +81 92 642 5482.
E-mail address: eriko@surg2.med.kyushu-u.ac.jp (E. Tokunaga).

0959-8049/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.ejca.2005.11.025

1. Introduction

Breast cancer is the most common cancer in women in the Western World, and the numbers breast cancer patients are also growing year by year in Japan.[1,2] Endocrine therapy was first introduced more than 100 years ago, however, it is still the most effective systemic treatment for patients with hormone receptor-positive breast cancer.

Tamoxifen is the most widely used selective estrogen receptor modulator (SERM) and it has been regarded as the gold standard endocrine therapy for hormone receptor-positive breast cancer.[3] Aromatase inhibitors (AIs) are new drugs which are used for endocrine treatment of post-menopausal breast cancer and they have demonstrated efficacy in patients with breast cancer resistant to anti-estrogens.[4] In addition, third-generation AIs, non-steroidal agents, letrozole and anastrozole, and the steroidal agent exemestane have all demonstrated both efficacy and safety advantages over tamoxifen. The increase in the use of the endocrine agents has resulted in the development of more strategies for the treatment of breast cancer. The major clinical problem in endocrine therapy is tumour resistance, either de novo or acquired during the treatment. About half of all estrogen receptor (ER)-positive tumours are responsive at first presentation to endocrine therapy, however, they eventually become resistant to it with the progression of the disease.[3]

Major clinical trials have shown that the ER status is the strongest and most reliable predictor of the response to endocrine therapy.[5] However, there are limitations in predicting the efficacy of endocrine therapy based on the hormone receptor expression alone. Progesterone receptor (PR) is an estrogen-regulated gene and the presence of PR is an indicator of a functional ER protein and a higher likelihood of a positive response to endocrine therapy.[6] However, about 30% of both ER- and PR-positive tumours remain resistant to endocrine therapy. These findings imply that other factors other than ER and PR are thus involved in resistance to endocrine therapy.

Recently, the cross-talk between signal transduction pathways and ER signalling has been focused on breast cancer etiology and progression.[7] This cross-talk, which occurs at multiple levels, has recently been shown to be associated with endocrine resistance.[8–12] Estrogen-activated membrane ER either directly or indirectly activates membrane tyrosine kinase receptors and this interaction leads to the activation of key secondary signalling messengers and downstream kinase pathways such as ERK/MAPK and PI3K/Akt. These kinases phosphorylate ER at key positions, and in turn, activate both nuclear ER transcriptional activity and promote ER-dependent transcription.[12]

Akt, which is also known as protein kinase B (PKB), is a serine/threonine protein kinase, which is activated by a variety of stimuli, through growth factor receptors, in a phosphoinositide-3-OH kinase (PI3-kinase)-dependent manner.[13] The disruption of normal Akt/PKB signalling occurs frequently in several human cancers, and this enzyme appears to play an important role in cancer progression and cell survival.[13] The mechanisms by which Akt promotes cell survival include phosphorylation of the pro-apoptotic proteins BAD, caspase-9, Forkhead transcription factors and IκB kinase α.[13] In addition, the mammalian target of rapamycin (mTOR) is a downstream effector of the PI3K/Akt signalling pathway that activates p70S6 kinase and 4E-binding protein-1, which in turn regulates the transition G1-S phase of the cell cycle. Breast cancer cell lines with a constitutively activated PI3K/Akt pathway due to HER2 overexpression and/or loss of the PTEN suppressor gene have been shown to be resistant to HER2-, EGFR-targeted therapies and to endocrine therapy with tamoxifen.[14] In addition, breast cancer cell lines with activated Akt are especially sensitive to mTOR antagonism.[15] Therefore, the PI3K/Akt signalling pathway currently attracts considerable attention as a new target for effective therapeutic strategies.

The activation of Akt/PKB has recently been shown to be positively associated with a worse outcome among endocrine-treated breast cancer patients.[16,17] In pre-menopausal patients who were treated with tamoxifen and/or goserelin, the patients with activated Akt were found to be more prone to suffering a relapse with distant metastasis.[16] On the other hand, in post-menopausal patients with a negative status of Akt showed a significant benefit from tamoxifen.[17] Recently, we have also reported Akt activation to be associated with a poor disease-free survival in cases with post-operative hormone therapy.[18] These findings suggest that the status of Akt activation could thus be used as a predictive marker for the sensitivity to endocrine therapy for breast cancer. However, the role of Akt in the resistance to endocrine therapy has not yet been clarified in metastatic breast cancer.

In the present study, we have investigated the relationship between Akt activation and the efficacy of endocrine therapy for metastatic breast cancer. We evaluated the activation of Akt by an immunohistochemical assessment of the expression of phosphorylated Akt (pAkt). pAkt positivity was thus found to be significantly associated with resistance to endocrine therapy. Our results suggest that: (1) Akt activation induces resistance to endocrine therapy; (2) Akt activation thus appears to be useful as a predictive marker of endocrine therapy and; (3) the inhibition of the Akt signalling pathway may improve the efficacy of endocrine therapy for metastatic breast cancer.

2. Patients and methods

2.1. *Patient population and tumour specimens*

A total 36 patients with metastatic breast carcinoma were investigated in this study, and all 36 patients had been treated with endocrine therapy at the Department of Surgery and Science, Kyushu University Hospital, or the Department of Breast Oncology, National Kyushu Cancer Center from 2002 to 2004. Primary human breast carcinoma specimens were obtained and subjected to pathological examinations and immunohistochemical analyses. Informed consent was obtained from all patients prior to tissue acquisition. Clinical data were obtained from medical records. The clinico-pathological features of these patients are described in Table 1.

Table 1 – Clinico-pathological features in the patients

Variables	Number
Menopausal status	
Pre-menopausal	10 (27.8%)
Post-menopausal	26 (72.2%)
Disease sites	
Bone	12
Lung	11
Lymph node	10
Soft tissue	6
Pleura	2
Liver	1
Others	2
Adjuvant therapy	
Chemotherapy	13
Endocrine therapy	9
Chemotherapy and endocrine therapy	9
None	5
ER	
Negative	3 (8.3%)
Positive	33 (91.7%)
PR	
Negative	9 (25.7%)
Positive	26 (74.3%)
Unknown	1
ER/PR	
Positive/negative	9 (25.7%)
Negative/positive	3 (8.6%)
Positive/positive	23 (65.7%)
Positive/unknown	1
HER2	
0, 1+	30 (83.3%)
2+	3 (8.3%)
3+	3 (8.3%)
pAkt	
Negative	24 (66.7%)
Positive	12 (33.3%)

2.2. *Assessment of the efficacy*

After initiating each endocrine therapy, the patients were assessed monthly to evaluate their clinical response. The response categories were defined according to World Health Organization criteria as a complete response (CR), partial response (PR), stable disease (SD), and progressive disease (PD). Both CR and PR were regarded as an objective clinical response. When CR, PR and SD longer than 6 months were obtained, the results were considered to demonstrate a clinical benefit.

2.3. *Immunohistochemistry*

Tissue samples were fixed by immersion in buffered formalin and then embedded in paraffin. Four-micron sections were placed onto charged slides and dried at 60 °C for 1 h. The sections were deparaffinized and hydrated in water. Immunostaining of these paraffin sections was performed using the Ventana Discovery™ automated staining instrument (Ventana Medical Instruments), and hematoxylin (Ventana Medical Instruments) was employed as a nuclear counterstained. Immunostaining was visualized with a streptoavidin peroxidase reaction using DAB as the chromogen (Ventana Medical Instruments). A negative-control reaction with no primary antibody was always performed alongside the reaction-containing sample. Immunostaining was evaluated without any knowledge of the clinical and pathological parameters.

2.4. *Predictive marker analysis*

As predictive markers for endocrine therapy, ER, PR and HER2 were analyzed by immunohistochemical staining. Monoclonal antibodies 6F11 and 1A6 (Ventana Medical Instruments, Tuscon, AR, USA) were used for ER and PR staining. For the HER2 evaluation, the monoclonal antibody, CB11 (Ventana Medical Instruments), was used.[18] ER and PR were considered to be positive if 10% or more of the nuclei in the invasive component of the tumour were stained.[19] HER2 was scored by the widely accepted criteria that assessed the intensity and completeness of membrane staining as previously described.[20,21] The intensity of membrane staining was evaluated according to the following criteria: score 0, none or up to 10% membrane staining; score 1+, partial and/or faint membrane staining present in more than 10% of tumour cells; score 2+, weak to moderate, complete membrane staining present in more than 10% of tumour cells; and score 3+, strong, complete membrane staining present in more than 10% of tumour cells. Scores 0 and 1+ were considered to be normal (i.e., negative for overexpression) and scores 2+ and 3+ were considered positive for HER2 overexpression.

2.5. *Evaluation of Akt activation*

The status of Akt activation was analyzed by the expression of phosophorylated Akt (pAkt). pAkt was detected using polyclonal antibodies against phosphorylated Ser 473 (Cell Signalling Technology, Beverly, MA, USA). A specimen was regarded as positive for pAkt when 10% or more of the cytoplasm of the tumour cells was positively stained.[18]

2.6. *Statistical analysis*

The associations between the categorical variables were assessed by means of the χ^2 tests. The cut-off for significance was set at $P < 0.05$.

3. Results

3.1. *Expression of hormone receptors and HER2 in breast carcinoma tissue specimens*

In this study, 36 primary breast carcinoma specimens, obtained from the patients with metastatic breast cancer, were evaluated. The expressions of estrogen receptor (ER), progesterone receptor (PR) and HER2 were investigated by immunohistochemistry (IHC). ER and PR were positive in 33 cases (91.7%) and 26 cases (74.3%), respectively. Both ER and PR were positive in 23 cases (65.7%). In most of these cases, HER2 was

negative (0 or 1+). HER2-positivity was 8.3% (3 cases) for HER2 2+ and was also 8.3% (3 cases) for HER2 3+ (Table 1).

3.2. *Expression of pAkt in primary breast cancer tissue specimens and the relationship between Akt activation and the HER2 status*

Of these 36 cases, 12 cases (33.3%) were regarded as positive for pAkt expression. Regarding the relationship between pAkt and HER2 expression, pAkt expression was positively correlated with HER2 expression ($P < 0.01$) even though the positivity of HER2 expression was low (Table 2). As a result, in HER2 2+ and 3+ tumours, Akt was more highly activated than HER2-negative tumours.

3.3. *Response to endocrine therapies*

The endocrine therapies received by these patients included the following; aromatase inhibitors (anastrozole or exemestane) in 23 patients, selective estrogen receptor modulator (SERM) (tamoxifen or tremifen) in 15 patients, luteinising hormone-releasing hormone (LHRH) agonist (Goselerin) with or without tamoxifen in seven patients, and medroxyprogesterone acetate (MPA) in one patient. The response data are shown in Table 3. Among 46 therapies in 36 patients, 5 CR and 11 partial responses were achieved. Objective response was observed in 16 cases (34.8%), while clinical benefit was observed in 27 (58.7%) therapies (5 CR, 11 partial responses and 11 long SD).

3.4. *Relationship between the clinical benefits and hormone receptor expression*

First of all, the relationship between clinical benefits and hormone receptor expression was examined. The clinical benefit rate was highest in ER-positive/PR-positive group. However, no statistical differences were seen in the clinical benefit in terms of the hormone receptor expression (Table 4).

Table 2 – Relationship between pAkt and the HER2 status

HER2	*n*	pAkt-negative	pAkt-positive	P-value
		(*n* = 24)	(*n* = 12)	
0, 1+	30	23 (76.7%)	7 (23.3%)	$P < 0.01$
2+	3	1 (20.0%)	2 (66.7%)	
3+	3	0 (0%)	3 (100%)	

3.5. *Relationships between the clinical benefits and the status of HER2 and pAkt*

We next investigated the relationship between HER2 status and clinical efficacy. As described in Table 5, the clinical benefit rate was lower in the HER2 2+ and 3+ groups ($P < 0.05$). As recent studies have suggested that high Akt activity in breast carcinoma is associated with poor prognosis in patients with adjuvant endocrine therapy, we, therefore, hypothesized that pAkt might be associated with poor response to endocrine therapy in metastatic breast cancer. As expected, pAkt positivity was significantly associated with ineffectiveness. In pAkt-positive patients, endocrine therapy had worse efficacy than in pAkt-negative patients ($P < 0.01$) (Table 5). pAkt positivity was also associated with poorer objective response ($P < 0.05$) (Table 6). In addition, the clinical benefit was the

Table 4 – Relationship between clinical benefit and hormone receptor expression

Variables	*n*	Clinical benefits		P-value
		Yes (*n* = 27)	No (*n* = 19)	
ER/PR				
Positive/negative	13	6 (46.2)	7 (53.8)	NS
Negative/positive	4	2 (50.0)	2 (50.0)	
Positive/positive	28	18 (64.3)	10 (35.7)	

Table 5 – Relationship between clinical benefit and the status of HER2 and pAkt

Variables	*n*	Clinical benefits		P-value
		Yes (*n* = 27)	No (*n* = 19)	
HER2				
0, 1+	39	26 (66.7)	13 (33.3)	$P < 0.05$
2+	3	1 (33.3)	2 (66.7)	
3+	4	0 (0)	4 (100.0)	
pAkt				
Negative	28	21 (75.0)	7 (25.0)	$P < 0.01$
Positive	18	6 (33.3)	12 (66.7)	
HER2/pAkt				
Negative/negative	27	20 (74.1)	7 (25.9)	$P < 0.01$
Positive/negative	1	1 (100.0)	0 (0)	
Negative/positive	12	6 (50.0)	6 (50.0)	
Positive/positive	6	0 (0)	6 (100.0)	

Table 3 – Endocrine therapies received by patients and their efficacies

Regimen	*n*	CR	PRes	SD	PD	OR (%)	Clinical benefit (%)
Aromatase inhibitors	23	4	5	5	9	9 (39.1)	14 (60.9)
LHRH agonist	7	0	2	1	4	2 (28.6)	3 (42.9)
SERM	15	1	4	4	6	5 (33.3)	9 (60.0)
Methylprogesterone acetate	1	0	0	1	0	0 (0)	1 (100)
Total	46	5	11	11	19	16 (34.8)	27 (58.7)

CR, complete response; PRes, partial response; SD, stable disease; PD, progressive disease; OR, objective response is CR + PRes; Clinical benefit is CR + PRes + SD.

Table 6 – Relationship between objective response and Akt activation

pAkt	CR, PR	SD, PD	P-value
	(*n* = 16, 34.8%)	(*n* = 30, 65.2%)	
Negative	13	15	P < 0.05
Positive	3	15	

CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease.

smallest in the both HER2 and pAkt-positive patients (P < 0.01) (Table 5).

3.6. *Relationships between the clinical benefits and the status of HER2 and pAkt by endocrine agents*

We next evaluated whether the kind of endocrine therapies influenced the association between efficacy and expression of HER2 or pAkt. We divided the different endocrine therapies into estrogen deprivation therapy such as aromatase inhibitors (AIs) or LHRH agonist and SERM (Table 7). In HER2 2+ and 3+ patients, the clinical benefit rate of AI or LHRH agonist was significantly lower than that in HER2 0 or 1+ patients (P < 0.01), although the number of the patients of HER2 2+ and 3+ was quite small (Table 7). On the other hand, no association was observed between the HER2 status and clinical efficacy of SERM, probably due to paucity of patients with HER2 overexpression. We then investigated the association between Akt activation and the clinical benefit by endocrine agents. In pAkt-positive patients, the clinical benefit rate of estrogen deprivation therapy, AI or LHRH, was significantly lower than that in pAkt-negative patients (P < 0.05). In addition, the clinical benefit of SERM tended to be smaller in the pAkt-positive patients (P = 0.09) (Table 7). These findings suggest that Akt activation induces endocrine resistance in metastatic breast cancer irrespective of the kind of endocrine agents that were administered.

Table 7 – Relationships between the clinical benefits and the status of HER2 and pAkt according to the endocrine agent

Variables	*n*	Clinical benefits		P-value
		Yes (*n* = 27)	No (*n* = 19)	
HER2				
AI or LH-RH agonist				
0, 1+	25	17 (68.0)	8 (32.0)	P < 0.01
2+	2	0 (0)	2 (100.0)	
3+	3	0 (0)	3 (100.0)	
SERM				
0, 1+	14	9 (64.3)	5 (35.7)	NS
3+	1	0 (0)	1 (100.0)	
pAkt				
AI or LH-RH agonist				
Negative	16	12 (75.0)	4 (25.0)	P < 0.05
Positive	14	5 (35.7)	9 (64.7)	
SERM				
Negative	11	8 (72.7)	3 (27.3)	P = 0.09
Positive	4	1 (25.0)	3 (75.0)	

4. Discussion

As far as we know, this is the first report to show an association between Akt activation and the efficacy of endocrine therapy in metastatic breast cancer. Akt/PKB is a serine/threonine kinase, which is a downstream effector of PI3K. Major functions of the PI3K/Akt signal pathway includes the promotion of growth-factor-mediated cell growth, proliferation, migration and survival.[13] Because the activation of the PI3K/Akt pathway induces resistance to the apoptotic response, the inhibition of this pathway is now considered to be a promising strategy to improve the effect of therapies for various kinds of cancers (reviewed in [22].)

Recent studies suggest high Akt activity in breast carcinoma to be associated with poor prognosis in patients with adjuvant endocrine therapy.[16–18] Perez-Tenorio and colleagues revealed that pAkt-positive patients were more prone to suffering a relapse with distant metastasis in a study of the pre-menopausal patients who were treated with tamoxifen and/or goserelin.[16] On the other hand, in a study of post-menopausal breast cancer patients, the benefit from tamoxifen was analyzed in estrogen receptor-positive patients.[17] Patients with a negative status of Akt showed a significant benefit from tamoxifen, whereas there was no significant benefit from tamoxifen in patients with positive Akt status.[17] In addition, we recently reported that pAkt positivity was associated with poor disease-free survival in cases with post-operative hormone therapy.[18]

We next investigated whether Akt activation had any impact on the response to endocrine therapy for metastatic breast cancer. In the present study, we analyzed 36 cases of metastatic breast cancer that had been treated with 46 endocrine therapies. In terms of the relationship between Akt activation and the efficacy of endocrine therapy, the clinical benefit rate was significantly lower in the pAkt-positive patients (P < 0.01) (Table 5). In addition, HER2 overexpression was associated with a lack of effectiveness of endocrine therapy, although the number of the patients with HER2 overexpression was too small (Table 5). However, this finding could have been expected based on previous experimental and clinical reports.[10,11,16,17,23]

We thereafter investigated whether the association between pAkt and resistance to endocrine therapy differed depending on the endocrine therapy agent. Up to now, the activation of Akt has been reported to be associated with resistance to anti-estrogen such as tamoxifen, however, the relationship between Akt activation and the effect of AIs or LHRH agonist has not yet been elucidated. Interestingly, pAkt-positivity or HER2 overexpression was significantly higher in the non-effective cases than that in effective cases with AIs or LHRH agonist, which are both types of estrogen depletion therapy (P < 0.01, P < 0.05) (Table 7). In experimental studies, estradiol has been shown to rapidly activate PI3K/Akt through the HER2 pathway[24] and a constitutively active Akt mutant mimics the effect of estrogen in the absence of the estrogen receptor ligand.[25] These results

suggest that breast cancer cells with activated Akt can survive under estrogen suppression by either AI or LHRH agonist. In terms of the relationship between HER2 overexpression and sensitivity to AI, a randomized trial of neoadjuvant therapy showed that, in a subset of ER-positive, epidermal growth factor receptor-positive and/or HER2-positive, letrozole was significantly more effective than tamoxifen.[19] However, in the metastatic setting, endocrine therapy has recently been shown to be less effective in patients with HER2-positive tumours irrespective of the drugs administered.[26] Our findings regarding the relationships between HER2 overexpression and sensitivity to estrogen depletion therapy are thus consistent with this meta-analysis in the metastatic setting.[26] In addition, we herein demonstrated that endocrine therapy is less effective in patients with Akt-activated breast cancer irrespective of the endocrine agents administered.

One possible mechanism for endocrine resistance in Akt-activated cells that we propose is illustrated in Fig. 1. When Akt is activated for any reason, it promotes the ER target gene expression even in the absence of estrogen or when being treated with tamoxifen. The estrogen independent tumour growth may also partly be associated with such resistance.

In this study, we have demonstrated that Akt/PKB activation was significantly associated with a poor response to endocrine therapy for metastatic breast cancer. The results of this study suggest that an inhibition of the Akt signalling pathway may improve the efficacy of the endocrine therapy for metastatic breast cancer. In fact, there are some currently ongoing or planned phase II/III clinical trials of endocrine therapy, either with or without signal transduction inhibitors, in locally advanced or metastatic breast cancer.[8] In combination with AIs and tamoxifen, monoclonal antibodies such as trastuzumab, and tyrosine kinase inhibitors such as gefinitib or lapanitib, and mTOR inhibitors, CCI-779 or RAD001 were recruited in these trials. Trastuzumab and tyrosine kinase inhibitors have a potency to inhibit Akt activity, and the inhibition of mTOR, which is downstream of Akt, can also lead to the inhibition of Akt signalling. The data obtained from these studies will hopefully lead to an improvement in the treatment of breast cancer patients.

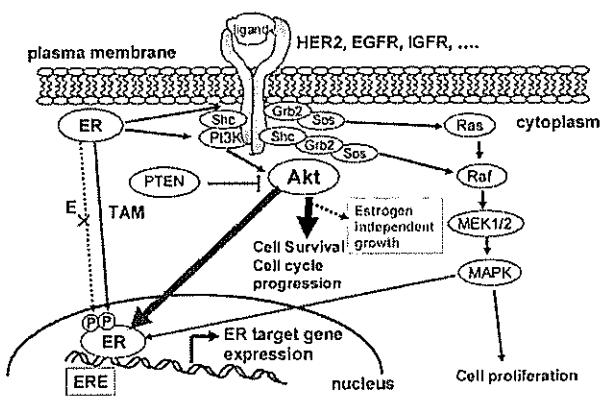

Fig. 1 – Possible mechanism for endocrine resistance in Akt-activated cells. In breast cancer cells, stimuli such as the activation of membrane tyrosine kinase and the loss of PTEN function are known to lead to Akt activation. Activated Akt promotes ER target gene expression even in the absence of estrogen (under treatment with aromatase inhibitors or LHRH agonists) or while being treated with tamoxifen, which induces cell growth. Estrogen independent tumour growth of Akt-activated cancer cells may partly be associated with such resistance.

In conclusion, this study suggests that pAkt may be a useful predictor of resistance to endocrine therapy for breast cancer, while also suggesting that the inhibition of Akt may increase the efficacy of endocrine therapy, although our study was small in scope and retrospective in design. Similar examinations in well-designed, larger-scale prospective studies should provide us more valuable findings in the future.

Conflict of interest statement

The authors of this paper have no financial or personal relationships that could bias this work.

Acknowledgements

We are grateful to Ms. Y. Kubota for her valuable technical assistance. This study was supported by grants from the Ministry of Education, Culture, Sports Science, and Technology of Japan.

REFERENCES

1. The Research Group for Population-based Cancer Registration in Japan. Cancer incidence and incidence rates in Japan in 1998: estimates based on data from 12 population-based cancer registries. *Jpn J Clin Oncol* 2003;33:241–5.
2. The Research Group for Population-based Cancer Registration in Japan. Cancer incidence Japan. *Gann Monogr Cancer Res* 1999;47:83–143.
3. Osborne CK. Tamoxifen in the treatment of breast cancer. *N Engl J Med* 1998;339:1609–18.
4. Janicke F. Are all aromatase inhibitors the same? A review of the current evidence. *Breast* 2004;13:S10–8.
5. Mouridsen HT, Rose C, Brodie AH, et al. Challenges in the endocrine management of breast cancer. *Breast* 2003;12:S2–S19.
6. Lapidus RG, Nass SJ, Davidson NE. The loss of estrogen and progesterone receptor gene expression in human breast cancer. *J Mammary Gland Biol Neoplasia* 1998;3:85–94.
7. Ali S, Coombes RC. Endocrine-responsive breast cancer and strategies for combating resistance. *Nat Rev Cancer* 2002;2:101–12.
8. Johnston SR. Combinations of endocrine and biological agents: present status of therapeutic and presurgical investigations. *Clin Cancer Res* 2005;11:889s–99s.
9. Johnston SR, Head J, Pancholi S, et al. Integration of signal transduction inhibitors with endocrine therapy: an approach to overcoming hormone resistance in breast cancer. *Clin Cancer Res* 2003;9:524S–32S.
10. Kurokawa H, Arteaga CL. Inhibition of erbB receptor (HER) tyrosine kinases as a strategy to abrogate anti-estrogen resistance in human breast cancer. *Clin Cancer Res* 2001;7:4436s–42s.. discussion 4411s–2s.

11. Kurokawa H, Arteaga CL. ErbB (HER) receptors can abrogate anti-estrogen action in human breast cancer by multiple signalling mechanisms. *Clin Cancer Res* 2003;9:511S–5S.
12. Schiff R, Massarweh SA, Shou J, et al. Cross-talk between estrogen receptor and growth factor pathways as a molecular target for overcoming endocrine resistance. *Clin Cancer Res* 2004;10:331S–6S.
13. Nicholson KM, Anderson NG. The protein kinase B/Akt signalling pathway in human malignancy. *Cell Signal* 2002;14:381–95.
14. Clark AS, West K, Streicher S, et al. Constitutive and inducible Akt activity promotes resistance to chemotherapy, trastuzumab, or tamoxifen in breast cancer cells. *Mol Cancer Ther* 2002;1:707–17.
15. Yu K, Toral-Barza L, Discafani C, et al. mTOR, a novel target in breast cancer: the effect of CCI-779, an mTOR inhibitor, in preclinical models of breast cancer. *Endocr Relat Cancer* 2001;8:249–58.
16. Perez-Tenorio G, Stal O. Activation of AKT/PKB in breast cancer predicts a worse outcome among endocrine treated patients. *Br J Cancer* 2002;86:540–5.
17. Stal O, Perez-Tenorio G, Akerberg L, et al. Akt kinases in breast cancer and the results of adjuvant therapy. *Breast Cancer Res* 2003;5:R37–44.
18. Tokunaga E, Kimura Y, Oki E, et al. Akt is frequently activated in HER2/neu-positive breast cancers and associated with poor prognosis among hormone treated patients. *Int J Cancer* 2006;118:284–9.
19. Ellis MJ, Coop A, Singh B, et al. Letrozole is more effective neoadjuvant endocrine therapy than tamoxifen for ErbB-1- and/or ErbB-2-positive, estrogen receptor-positive primary breast cancer: evidence from a phase III randomized trial. *J Clin Oncol* 2001;19:3808–16.
20. Jacobs TW, Gown AM, Yaziji H, et al. Specificity of Hercep Test in determining HER-2/neu status of breast cancers using the United States Food and Drug Administration-approved scoring system. *J Clin Oncol* 1999;17:1983–7.
21. Seidman AD, Fornier MN, Esteva FJ, et al. Weekly trastuzumab and paclitaxel therapy for metastatic breast cancer with analysis of efficacy by HER2 immunophenotype and gene amplification. *J Clin Oncol* 2001;19:2587–95.
22. Thompson JE, Thompson CB. Putting the rap on Akt. *J Clin Oncol* 2004;22:4217–26.
23. Knuefermann C, Lu Y, Liu B, et al. HER2/PI-3K/Akt activation leads to a multidrug resistance in human breast adenocarcinoma cells. *Oncogene* 2003;22:3205–12.
24. Stoica GE, Franke TF, Wellstein A, et al. Estradiol rapidly activates Akt via the ErbB2 signalling pathway. *Mol Endocrinol* 2003;17:818–30.
25. Stoica GE, Franke TF, Moroni M, et al. Effect of estradiol on estrogen receptor-alpha gene expression and activity can be modulated by the ErbB2/PI 3-K/Akt pathway. *Oncogene* 2003;22:7998–8011.
26. De Laurentiis M, Arpino G, Massarelli E, et al. A meta-analysis on the interaction between HER-2 expression and response to endocrine treatment in advanced breast cancer. *Clin Cancer Res* 2005;11:4741–8.

Phase I study of S-1 and biweekly docetaxel combination chemotherapy for advanced and recurrent gastric cancer

IKUO TAKAHASHI[1], YASUNORI EMI[1], YOSHIHIRO KAKEJI[1], ERIKO TOKUNAGA[1], SHIN USHIRO[1], EIJI OKI[1], MASAYUKI WATANABE[1], HIDEO BABA[2] and YOSHIHIKO MAEHARA[1]

[1]Department of Surgery and Science, Graduate School of Medical Sciences, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582; [2]Department of Gastroenterological Surgery, Graduate School of Medical Sciences, Kumamoto University, 1-1-1 Honjo, Kumamoto 860-8556, Japan

Received August 16, 2005; Accepted October 14, 2005

Abstract. A phase I study of S-1 and biweekly docetaxel (DOC) combination therapy was conducted to determine the maximum tolerated dose (MTD) and pharmacokinetic parameters. Fourteen patients with advanced or recurrent gastric cancer were analyzed. The treatment consisted of S-1 [body surface area (BSA) <1.25 m^2:80 mg/day, 1.25≤ BSA <1.50 m^2: 100 mg/day, 1.50 m^2≤ BSA; 120 mg/day, orally, day 1-14) and DOC (30-40 mg/m^2/day, intravenously, day 1 and 15], which were repeated as often as possible every four weeks. Pharmacokinetic analysis was done at DOC 40 mg/m^2/day. Initially, patients were administered S-1 and 40 mg/m^2/day of DOC, and DOC 40 mg/m^2/day was considered as MTD. In detail, one patient developed neutropenia (grade 4, G4), and two other patients had no day 15 DOC administration because of neutropenia (grade 3, G3). When S-1 and 35 mg/m^2/day of DOC were administered to three patients, no adverse reactions were noted. In six patients treated with S-1 and 30 mg/m^2/day of DOC, one patient developed neutropenia (G4), and another patient developed diarrhea (G3) and anorexia (G3). The rest of this cohort showed no adverse reactions. Although 5-fluorouracil and gimeracil concentrations remained high under impaired renal function, no pharmacokinetic interactions appeared between S-1 and DOC under normal renal function. The dose limiting toxicity of a combination of S-1 and biweekly DOC was leukopenia and neutropenia. The recommended dose for this combination in phase II study is DOC 35 mg/m^2/day.

Introduction

S-1 is a novel oral anticancer drug, which was developed based on the biochemical modulation of tegafur (FT) by 5-chloro-2,4-dihydroxypyridine (gimeracil, CDHP) and potassium oxonate (oteracil, oxo) in a molar ratio of 1:0.4:1 (1-3). FT is a prodrug of 5-fluorouracil (5-FU), an active drug against various forms of gastrointestinal malignancy. 5-FU is degraded to α-fluoro-ß-alanine by dihydropyrimidine dehydrogenase (DPD), which is produced in various organs, including tumor tissue. CDHP strongly inhibits DPD, which results in a prolonged increased concentration of 5-FU in the plasma (4). Oxo inhibits phosphorylation of 5-FU to 5-fluorouridine-5'-monophosphate (5). As oxo is distributed in the gastrointestinal tract after oral administration, it possibly decreases 5-FU-induced gastrointestinal tract toxicity (5). Thus, S-1 was designed both to increase antitumor activity and to reduce drug-induced adverse gastrointestinal adverse reaction.

Independent phase II studies of S-1, including gastric cancer patients without prior chemotherapy, give an excellent response rate and survival (6-8). The response rate using S-1 alone is comparable to or better than the response rate in combination studies such as FAMTX or ECF, which has been mainly used in Europe or the United States (9-12). S-1 has been in common use in clinical practice since March 1999 in Japan against advanced and recurrent gastric cancer. Clinical information on this drug has accumulated. We previously summarized recent clinical data of 29 patients with advanced or recurrent gastric cancer and, as expected, a high response rate and good survival were confirmed in patients without prior chemotherapy (13). However, in patients who had received chemotherapy, the response rate was 12.5%. Today, S-1 is a main drug for the treatment of advanced or recurrent gastric cancer, especially in first-line chemotherapy. However, the questions arise as to which is the best chemotherapy regimen for second-line chemotherapy, and whether combination therapy of S-1 is superior to S-1 monotherapy. Thus, a new combination therapy of S-1 is worth exploring.

Docetaxel (DOC) is a semi-synthetic taxane prepared from a non-cytotoxic precursor extracted from the needles of the European yew tree *Taxus baccata*. Docetaxel accelerates microtubule assembly from tubulin, and blocks depolymerization of microtubules. Stable microtubules result in cell death. As a single agent, taxanes are currently the most widely administered agents for metastatic breast cancer after anthracycline treatment (14). DOC is also active against gastric cancer. Independent

Correspondence to: Dr Ikuo Takahashi, Department of Surgery and Science, Graduate School of Medical Sciences, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan
E-mail: takahashii@matsuyama.jrc.or.jp

Key words: gastric cancer, S-1, docetaxel, phase I study

Table I. Patient characteristics (n=14).

Covariate		No. of patients
Gender		
Male		13
Female		1
Age (years old)		58 (41-72)
Performance status		
0		10
1		4
Type of disease		
Recurrence		7
Inoperable		3
Postoperation with residual tumor		4
Histological type		
Differentiated		6
Undifferentiated		8
Treatment course	24 courses	[1-4 courses for each pt]
Level 1 (n=5)	9 courses	[1-4 courses for each pt]
Level 0 (n=3)	7 courses	[1-4 courses for each pt]
Level -1 (n=6)	8 courses	[1-2 courses for each pt]
Calculated creatinine clearance (ml/min)	81.3	(33.9-129.8)

pt, patient.

Table II. Hematological toxicity.

	NCI-CTC grade				
	1	2	3	4	% grade ≥3
Level 1 (n=5)					
Leukopenia	1	1	1	1	40
Neutropenia			2	1	60
Anemia		3	1		20
Thrombocytopenia	1				0
Level 0 (n=3)					
Leukopenia		1			0
Neutropenia		1			0
Anemia		1			0
Thrombocytopenia					0
Level -1 (n=6)					
Leukopenia	1	1	3		50
Neutropenia		1	2	1	50
Anemia		4			0
Thrombocytopenia					0

NCI-CTC grade, National Cancer Institute-Common Toxicity Criteria grade.

phase II studies in Japan showed that the response rate is 23.7% (15,16).

Recent reports of docetaxel-based combination therapy showed high response rates as a first line for gastric cancer patients (17,18). We focused on DOC as a candidate for combination therapy with S-1, and we made a phase I study of S-1 and biweekly DOC combination therapy, to find the maximum tolerated dose (MTD) and recommended dose in a phase II clinical trial.

Patients and methods

Eligibility. Patients with advanced or recurrent gastric cancer were eligible for this study. This study started after obtaining approval from the institutional review board. Disease characteristics included the following criteria: i) histologically or cytologically proved gastric cancer, ii) measurable or evaluable lesions, iii) no prior chemotherapy (history of postoperative adjuvant chemotherapy was allowed), and iv) adjuvant therapy (including chemotherapy and immunotherapy) must be finished at least four weeks before the combination therapy starts. Patient characteristics included the following criteria: i) age of ≥20 and <75 years, ii) an Eastern Cooperative Oncology Group performance status of ≤2, iii) adequate hematopoietic function (4000/mm^3≤ white blood cell ≤12000/mm^3, neutrocyte, ≥2000/ mm^3, platelet ≥10x10^4/mm^3, hemoglobin ≥9.0 g/dl), iv) adequate hepatic function [total bilirubin ≤1.5 mg/dl, transaminase ≤2 times institutional normal upper limit (if caused by liver metastases, transaminase ≥2 times may be allowed based on the doctor's judgement)], v) serum creatinine ≤ institutional normal upper limit, blood urea nitrogen ≤25 mg/dl, vi) adequate cardiac function, vii) neither brain metastases nor history of brain metastases, and viii) before treatment, written informed consent must be obtained from the patients. As complimentary data about renal function, creatinine clearance was calculated based on a formula described elsewhere (19).

From March 2001 to June 2003, 17 patients were enrolled in this study. Three patients were excluded because 1 of them showed marked disease progression before initiation of the treatment and 2 of them took half of the indicated dose of S-1 during the treatment. Thus, a total of 14 patients were evaluated.

Dose and drug administration. S-1 was administered orally in the morning and evening on days 1-14 according to the body surface area (BSA); BSA <1.25 m^2; 80 mg/day, 1.25≤ BSA <1.50 m^2; 100 mg/day, 1.50 m^2≤ BSA; 120 mg/day). DOC was diluted in normal saline and was administered with an infusion pump for 1 h on days 1 and 15. As a premedication, 8 mg of dexamethasone was administered intravenously, 0.5 h before; and 4 mg orally, 12, 24, 36 and 48 h after the start of DOC administration. One course was 28 days, and was repeated as often as possible. Treatment continued unless disease progressed, unacceptable toxicity occurred or the patient refused further treatment. The dose of DOC started at

Table III. Non-hematological toxicity

	NCI-CTC grade				
	1	2	3	4	% grade ≥3
Level 1 (n=5)					
Anorexia	1		1		20
Diarrhea			1		20
General fatigue	1				0
Skin eruption	1				0
Level 0 (n=3)					
Anorexia	1				0
Nausea, vomiting	1				0
Stomatitis	1				0
General fatigue	1				0
Alopecia	1				0
Level -1 (n=6)					
Anorexia	2		1		16.7
Diarrhea	1	1		1	16.7
Stomatitis	1				0
General fatigue	2	1			0
Alopecia	4				-
Headache	2				0
Abdominal pain		1			0

NCI-CTC grade, National Cancer Institute-Common Toxicity Criteria grade.

40 mg/m^2 (Level 1). Doses of 35 mg/m^2 (Level 0) and 30 mg/m^2 (Level -1) were also evaluated because the dose limiting toxicity (DLT) was at Level 1.

DLT, MTD and dose escalation schedule. Toxicity was evaluated according to the National Cancer Institute (NCI) common toxicity criteria. The DLT was defined as: i) grade 4 leukopenia or neutropenia lasting longer than three days despite the use of granulocyte-colony stimulating factor (G-CSF), ii) grade 3 neutropenia with a fever of >38˚C lasting longer than three days despite the use of G-CSF, iii) grade 4 thrombocytopenia or grade 3 thrombocytopenia with bleeding tendency, and iv) grade 3-4 non-hematological toxicity except nausea, vomiting or alopecia.

In an initial study, three patients at one dose level were evaluated: i) the dose was defined as MTD when all patients developed DLT; ii) when one or two of three patients developed DLT, three other patients were enrolled; iii) when more than three of six patients developed DLT, the dose was defined as MTD; iv) when fewer than two of six patients developed DLT, the dose was increased to the next step.

Pharmacokinetic study design. Pharmacokinetic study was conducted for three patients on the first day of treatment at dose level 1 (S-1 at the fixed dose described above, and DOC 40 mg/m^2). S-1 and DOC administration started simultaneously, and heparinized blood samples to test for 5-FU, FT, CDHP and oxo were taken from the patients before and 1, 2, 4 and 8 h after administration. Heparinized blood samples to test for DOC were taken before and 0.5, 1, 1.5, 2, 3, 4, 5 and 8 h after administration. Immediately, the blood samples were cooled in ice and then centrifuged at 3000 round per minute for 15 min, and the separated serums were stored at -80˚C until assay. The plasma levels of FT, 5-FU, CDHP, oxo and DOC concentration were measured as described elsewhere (20,21). WinNonlin ver.3.0 (Phasight Co.) software was used to calculate the pharmacokinetic parameters such as maximum plasma concentration (Cmax), time to maximum plasma concentration (Tmax), area under the plasma concentration-versus-time curve from time zero to infinity (AUC 0-∞), and plasma elimination half-life (T 1/2).

Independently from this phase I study, three patients with advanced or recurrent gastric cancer were treated with docetaxel monotherapy biweekly (40 mg/m^2). After informed consent was obtained, blood samples were taken at the same times as used in the phase I study. The plasma docetaxel concentration was measured by using the same method.

Results

Patients characteristics, toxicity and DLT. Table I lists patient characteristics. The performance status was 0 in 10 patients, and the median age was 58 years old. Twenty four courses were conducted, with a mean of 1.7 courses for each patient (range 1-4 courses). The creatinine clearance was >50 ml/min except for patient 1 in the pharmacokinetic study. Tables II and III list hematological and non-hematological toxicity profiles.

This study started with a cohort of three patients at DOC dose 40 mg/m^2 (Level I). Leukopenia and neutropenia were found as DLT, and Level 1 (40 mg/m^2) as MTD. In detail, one patient developed grade 4 neutropenia and another patient could not receive day 15 DOC administration due to prolonged grade 3 leukopenia and neutropenia; the third patient developed no adverse reactions. Two additional patients were enrolled in Level 1; one of these patients also could not receive day 15 DOC administration because of grade 3 neutropenia.

In the second step, we evaluated the DOC dose at 30 mg/m^2 (Level -1) as the safety of the registered patients was the highest priority. Among the first three patients in this cohort, one patient completed two courses of treatment, one patient developed grade 4 leukopenia and neutropenia and the third patient discontinued treatment because this patient developed grade 3 anorexia and grade 3 diarrhea. As the latter two patients were considered to show DLT, an additional three patients were enrolled in this cohort. Two of these patients developed grade 3 leukopenia and neutropenia, and the third patient developed grade 2 diarrhea, abdominal pain and grade 1 headache: no DLT appeared during the treatment. Thus, 30 mg/m^2 (Level -1) was not considered as DLT.

In the third step, three patients received S-1 and DOC 35 mg/m^2 (Level 0), to confirm if DOC at 30 mg/m^2 (Level -1) or DOC at 35 mg/m^2 (Level 0) can be a recommended dose. Although grade 2 leukopenia, neutropenia and anemia developed in 1 patient, grade 3 and 4 hematological toxicity were not noted. When considering non-hematological toxicity, only grade 1 anorexia, nausea, vomiting, stomatitis, general

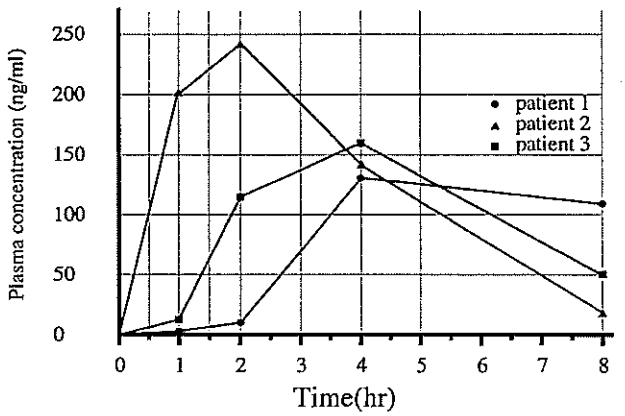

Figure 1. Plasma 5-fluorouracil concentration after administration of combined S-1 and docetaxel (DOC) (S-1, 50 mg/body, orally; DOC, 40 mg/m², 1 h infusion, intravenously).

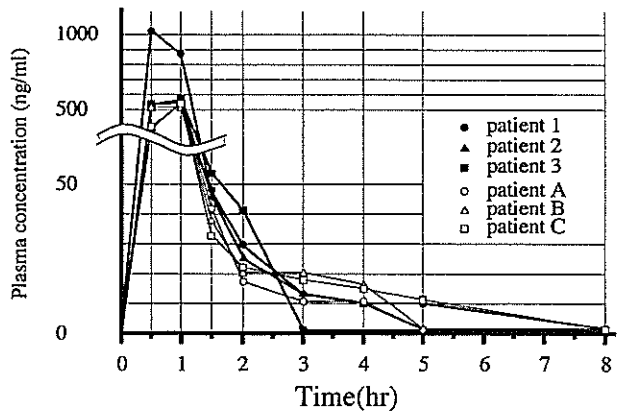

Figure 2. Plasma docetaxel (DOC) concentration after administration of DOC alone or DOC and S-1 combination. (S-1, 50 mg/body, orally; DOC, 40 mg/m², 1 h infusion, intravenously). Patients 1-3 were treated with a combination of S-1 and DOC, whereas patients A-C were treated with DOC monotherapy.

fatigue and alopecia were noted. Thus, DOC at 35 mg/m² (Level 0) was considered a recommended dose.

Pharmacokinetics. A pharmacokinetic study was conducted with three patients on the first day of treatment at dose level 1 (S-1 orally at the dose stated in the Patients and methods section, and DOC at 40 mg/m² intravenously for 1 h). Fig. 1 shows 5-FU concentrations in combined therapy (patients 1-3). Fig. 2 shows DOC concentrations in patients treated with combined therapy (patients 1-3), as well as in patients treated with DOC at 40 mg/m² monotherapy (patients A-C). Table IV shows the Cmax, Tmax, AUC 0-∞ and T1/2. Although the Cmax of 5-FU was from 132.6 to 244.4 ng/ml, the 5-FU concentration was maximum at 2-4 h after administration. One patient with impaired renal function (patient 1) showed a prolonged 5-FU concentration plateau and a longer half-life of 5-FU.

In terms of DOC pharmacokinetics, plasma DOC concentration in the Level 1 combination therapy group was maximum at 0.5-1 h after the start of administration (i.e., 0.5 h before or at the end of administration), and then decreased rapidly. The change in plasma DOC concentration in the DOC monotherapy group showed the same pharmacokinetic profile.

Table IV. Pharmacokinetic parameters after administration of S-1 and DOC (40 mg/m²) combination therapy (patients 1-3) and DOC monotherapy (patients A-C).

	Cmax (ng/ml)	Tmax (h)	AUC 0-∞ (ng. h/ml)	T 1/2 (h)
5-FU				
Patient 1	132.6	4	2737	13.6
Patient 2	244.4	2	1072	1.31
Patient 3	158.0	4	941	2.42
FT				
Patient 1	3235.1	4	33295	5.99
Patient 2	2008.6	1	10610	3.91
Patient 3	1859.8	2	16658	6.88
CDHP				
Patient 1	294.3	4	2054	3.89
Patient 2	428.6	1	1380	2.10
Patient 3	259.4	2	1559	3.30
Oxo				
Patient 1	71.7	4	506	3.59
Patient 2	112.8	1	495	2.50
Patient 3	38.3	2	771	13.6
DOC				
Patient 1	1030	0.5	1055.6	1.87
Patient 2	554	1	625.4	1.53
Patient 3	569	1	660.0	1.18
DOC				
Patient A	544	1	490.7	1.85
Patient B	510	0.5	655.3	3.22
Patient C	517	1	533.2	2.99

Cmax, maximum plasma concentration; Tmax, time to maximum plasma concentration; AUC 0-∞, area under the plasma concentration-versus-time curve from time zero to infinity; T1/2, plasma elimination half-life.

Discussion

In Japan, gastric cancer still remains most frequent malignancy. Despite of the advance in early detection of this disease and surgical improvement, the survival of patients with recurrent and advanced gastric cancer is unsatisfactory. The Japan Gastric Cancer Association issued the first edition of gastric cancer treatment guidelines in March 2001 to provide a common basis of understanding of the extent of disease and selection of proper treatment (22). This guideline did not mention particular regimen of chemotherapy for advanced and recurrent gastric cancer, but it stated that 5-FU and cisplatin may be important drugs. Thus, the standard regimen has not been established.

S-1 is synthesized in Japan and has been a key drug in the treatment of advanced and recurrent gastric cancer in Japan because of a high response rate (7,8,13). In this study, we investigated the safety and pharmacokinetic profiles of a combination therapy of S-1 and biweekly DOC. As a monotherapy, the DLT of DOC is leukopenia and neutropenia (15,16). Although, in patients in Europe and in the United States, the DLT of S-1 is diarrhea (23), the DLT of S-1 in Japanese patients is mainly haematological and stomatitis and diarrhea is mild (6-8). As expected, in the S-1 and biweekly DOC regimen, the DLT was leukopenia and neutropenia in this study. Although the toxicity profile of both drugs is similar in Japanese patients, we thought it rational to examine this combination for the following reasons: i) the mechanism of antitumor activity of S-1 and DOC is completely different, ii) DOC is beneficial for gastric cancer patients who have been previously treated and for patients as a first-line therapy (15,16), and iii) the resistance of 5-FU is overcome by DOC *in vitro* (24). Our previous *in vivo* therapeutic experiment that used gastric cancer xenografts showed that S-1 (day 1-14 administration) and DOC (day 1 or day 8 intravenous administration) were synergistic (25), although schedule dependency between paclitaxel and 5-fluorouracil *in vitro* has been reported (26). Thus, we scheduled days 1-14 of administration with S-1 with days 1 and 15 of administration with DOC.

The combination therapy of DOC and continuous 5-FU has showed no pharmacokinetic interaction between the two drugs (27,28). As the pharmacokinetics of S-1 is similar to that of continuous 5-FU intravenous infusion (29), S-1 may not interact with DOC unless the 5-FU concentration remains the same as expected. One study showed that pharmacokinetic parameters of 5-FU in Japanese patients were Cmax, 128.5 ng/ml; Tmax, 3.5 h; AUC 0-14, 724 ng.h/ml; and T1/2, 1.9 h (29). In our study, the AUC of 5-FU in patients 2 and 3 were similar to these data. Patient 1, which developed grade 4 leukopenia and neutropenia, showed a larger AUC 0-∞ of 5-FU and CDHP. The calculated creatinine clearance was 33.9 ml/min. The different pharmacokinetic profile may be partly explained by impaired renal function. CDHP, excreted in the kidney, inhibits DPD, the catabolic enzyme of 5-FU, and results in a prolonged and higher 5-FU concentration in the plasma. Impaired renal function is directly connected to an elevated level of CDHP, which results in a prolonged plasma 5-FU plateau level. The results of a postmarketing survey of S-1 in Japan support this hypothesis (30). This survey monitored the toxicity profile and estimated creatinine clearance and showed that patients with a lower creatinine clearance frequently experience severe adverse reactions. In terms of DOC, hepatic metabolism and biliary excretion is the major pathway of DOC elimination (31). Our data showed that the plasma concentration of DOC is maximum just after drug administration, and the same pharmacokinetic profiles were confirmed with or without combination with S-1.

In conclusion, patients should be strictly screened for impaired renal function based on creatinine clearance. Although careful blood count monitoring should be used, a combination therapy of S-1 and biweekly DOC is worth investigating. The recommended dose for this combination is 35 mg/m^2/day DOC on days 1 and 15.

References

1. Takechi T, Nakano K, Uchida J, Mita A, Toko K, Takeda S, Unemi N and Shirasaka T: Antitumor activity and low intestinal toxicity of S-1, a new formulation of tegafur, in experimental tumor models in rats. Cancer Chemother Pharmacol 39: 205-211, 1997.
2. Fukushima M, Shimamoto Y, Kato T, Uchida J, Yonekura R, Ohshimo H and Shirasaka T: Antitumor activity and toxicity of S-1, an oral combination of tegafur and two biochemical modulators, compared with continuous infusion of 5-fluorouracil. Anticancer Drugs 9: 817-823, 1998.
3. Fukushima M, Satake H, Uchida J, Shimamoto Y, Kato T, Takechi T, Okabe H, Fujioka A, Nakano K, Ohshimo H, Takeda S and Shirasaka T: Preclinical antitumor efficacy of S-1: a new oral formulation of 5-fluoruoracil on human tumor xenografts. Int J Oncol 13: 693-698, 1998.
4. Tatsumi K, Fukushima M, Shirasaka T and Fujii S: Inhibitory effects of pyrimidine, barbituric acid and pyridine derivatives on 5-fluorouracil degradation in rat liver extracts. Jpn J Cancer Res 78: 748-755, 1987.
5. Shirasaka T, Shimamoto Y and Fukushima M: Inhibition by oxonic acid of gastrointestinal toxicity of 5-fluorouracil without loss of its antitumor activity in rats. Cancer Res 53: 4004-4009, 1993.
6. Sugimachi K, Machara Y, Horikoshi N, Shimada Y, Sakata Y, Mitachi Y and Taguchi T: An early phase II study of oral S-1, a newly developed 5-fluorouracil derivative for advanced and recurrent gastrointestinal cancers. Oncology 57: 202-210, 1999.
7. Koizumi W, Kurihara M, Nakano S and Hasegawa K: Phase II study of S-1, a novel oral derivative of 5-fluorouracil, in advanced gastric cancer. Oncology 58: 191-197, 2000.
8. Sakata Y, Ohtsu A, Horikoshi N, Sugimachi K, Mitachi Y and Taguchi T: Late phase II study of novel oral fluoropyrimidine anticancer drug S-1 (1 M tegafur - 0.4 M gimestat - 1 M otastat potassium) in advanced gastric cancer patients. Eur J Cancer 34: 1715-1720, 1998.
9. Wils JA, Klein HO, Wagener DJ, Bleiberg H, Reis H, Korsten F, Conroy T, Fickers M, Leyvraz S, Buyse M and Duez N for the European Organization for Research and Treatment of Cancer, Gastrointestinal Tract Cooperative Group: Sequential high-dose methotrexate and fluorouracil combined with doxorubicin - a step ahead in the treatment of advanced gastric cancer: a trial of the EORTC Gastrointestinal Tract Cooperative Group. J Clin Oncol 9: 827-831, 1991.
10. Webb A, Cunningham D, Scarffe JH, Harper P, Norman A, Joffe JK, Hughes M, Mansi J, Findlay M, Hill A, Oates J, Nicolson M, Hickish T, O'Brien M, Iveson T, Watson M, Underhill C, Wardley A and Meehan M: Randomized trial comparing epirubicin, cisplatin and fluorouracil versus fluorouracil, doxorubicin and methotrexate in advanced esophagogastric cancer. J Clin Oncol 15: 261-267, 1997.
11. Waters JS, Norman A, Cunningham D, Scarffe JH, Webb A, Harper P, Joffe JK, Mackean M, Mansi J, Leahy M, Hill A, Oates J, Rao S, Nicolson M and Hickish T: Long-term survival after epirubicin, cisplatin and fluorouracil for gastric cancer: results of a randomized trial. Br J Cancer 80: 269-272, 1999.
12. Kelsen D, Atiq OT, Saltz L, Niedzwiecki D, Ginn D, Chapman D, Heelan R, Lightdale C, Vinciguerra V and Brennan M: FAMTX versus etoposide, doxorubicin and cisplatin: a random assignment trial in gastric cancer. J Clin Oncol 10: 541-548, 1992.
13. Takahashi I, Kakeji Y, Emi Y, Sakurai M, Yonemura Y, Kimura Y and Maehara Y: S-1 in the treatment of advanced and recurrent gastric cancer: current status and future prospects. Gastric cancer 6 (suppl 1): S28-S33, 2003.
14. Valero V, Holmes FA, Walters RS, Theriault RL, Esparza L, Fraschini G, Fonseca GA, Bellet RE, Buzdar AU and Hortobagyi GN: Phase II trial of docetaxel: a new, highly effective antineoplastic agent in the management of patients with anthracycline-resistant metastatic breast cancer. J Clin Oncol 13: 2886-2894, 1995.
15. Taguchi T, Sakata Y, Kanamaru R, Kurihara M, Suminaga M, Ota J and Hirabayashi N: Late phase II clinical study of RP56976 (Docetaxel) in patients with advanced/recurrent gastric cancer: a Japanese cooperative study group trial (group A). Jpn J Cancer Chemother 25: 1915-1924, 1998.
16. Mai M, Sakata Y, Kanamaru R, Kurihara M, Suminaga M, Ota J, Hirabayashi N, Taguchi T and Furue H: A late phase II clinical study of RP56976 (docetaxel) in patients with advanced or recurrent gastric cancer: a cooperative study group trial (group B). Jpn J Cancer Chemother 26: 487-496, 1999.

17. Park YH, Ryoo BY, Choi SJ and Kim HT: A phase II study of capecitabine and docetaxel combination chemotherapy in patients with advanced gastric cancer. Br J Cancer 90: 1329-1333, 2004.
18. Roth AD and Ajani J: Docetaxel-based chemotherapy in the treatment of gastric cancer. Ann Oncol 14 (suppl 2): ii41-ii44, 2003.
19. Cockcroft DW and Gault MH: Prediction of clearance from serum creatinine. Nephron 16: 31-41, 1976.
20. Matsushima E, Yoshida K, Kitamura R and Yoshida K: Determination of S-1 (combined drug of tegafur, 5-chloro-2,4-dihydroxypyridine and potassium oxonate) and 5-fluorouracil in human plasma and urine using high-performance liquid chromatography and gas chromatography-negative ion chemical ionization mass spectrometry. J Chromatogr 691: 95-100, 1997.
21. Vergniol JC, Bruno R, Montay G and Frydman A: Determination of taxotere in human plasma by a semi-automated high-performance liquid chromatographic method. J Chromatogr 582: 273-278, 1992.
22. Nakajima T: Gastric cancer treatment guidelines in Japan. Gastric Cancer 5: 1-5, 2002.
23. Hoff PM, Saad ED, Ajani JA, Lassere Y, Wenske C, Medgyesy D, Dwivedy S, Russo M and Pazdur R: Phase I study with pharmacokinetics of S-1 on an oral daily schedule for 28 days in patients with solid tumors. Clin Cancer Res 9: 134-142, 2003.
24. Hill BT, Whelan RD, Shellard SA, McClean S and Hosking LK: Differential tumor cell lines and certain drug resistant sublines *in vitro*. Invest New Drugs 12: 169-182, 1994.
25. Takahashi I, Emi Y, Kakeji Y, Uchida J, Fukushima M and Maehara Y: Increased antitumor activity in combined treatment TS-1 and docetaxel - a preclinical study using gastric cancer xenografts. Oncology 68: 130-137, 2005.
26. Grem JL, Nguyen D, Monahan BP, Kao V and Geoffroy FJ: Sequence-dependent antagonism between fluorouracil and paclitaxel in human breast cancer cells. Biochem Pharmacol 58: 477-486, 1999.
27. Lortholary A, Maillard P, Delva R, Boisdron-Celle M, Perard D, Vernillet L, Besenval M and Gamelin E: Docetaxel in combination with 5-fluorouracil in patients with metastatic breast cancer previously treated with anthracycline-based chemotherapy: a phase I, dose-finding study. Eur J Cancer 36: 1773-1780, 2000.
28. Ando M, Watanabe T, Sasaki Y, Ying DF, Omuro Y, Katsumata N, Narabayashi M, Tokue Y, Fujii H, Igarashi T, Wakita H, Ohtsu T, Itoh K, Adachi I and Taguchi T: A phase I trial of docetaxel and 5-day continuous infusion of 5-fluorouracil in patients with advanced or recurrent breast cancer. Br J Cancer 77: 1937-1943, 1998.
29. Hirata K, Horikoshi N, Aiba K, Okazaki M, Denno R, Sasaki K, Nakano Y, Ishizuka H, Yamada Y, Uno S, Taguchi T and Shirasaka T: Pharmacokinetic study of S-1, a novel oral fluoropyrimidine, uracil antitumor drug. Clin Cancer Res 5: 2000-2005, 1999.
30. Nagashima F, Ohtsu A, Yoshida S and Ito K: Japanese nationwide post-marketing survey of S-1 in patients with advanced gastric cancer. Gastric Cancer 8: 6-11, 2005.
31. Marre F, Sanderink GJ, De Sousa G, Gaillard C, Martinet M and Rahmani R: Hepatic biotransformation of docetaxel (taxotere) *in vitro*: involvement of the CYP3A subfamily in humans. Cancer Res 56: 1296-1302, 1996.

S-1の基礎と臨床
結腸・直腸癌

術後補助化学療法におけるフッ化ピリミジン系薬剤の有用性に関する比較臨床試験

—治癒切除直腸癌に対するUFT療法とS-1療法との比較検討—

沖　英次[*1]　掛地　吉弘[*1]　吉田倫太郎[*1]　池田　圭介[*1]　西田康二郎[*1]
古賀　聡[*1]　江頭　明典[*1]　徳永えり子[*1]　森田　勝[*1]　馬場　秀夫[*2]
前原　喜彦[*1]

〔*Jpn J Cancer Chemother* 33(*Suppl* I): 138-143, *June*, 2006〕

A Randomized Controlled Trial to Evaluate the Effect of Adjuvant Oral Fluoropyrimidine Derivative Therapy after Curative Resection for Stage II/III Rectal Cancer—Adjuvant Chemotherapy Trial of S-1 for Rectal Cancer (ACTS-RC): Eiji Oki[*1], Yoshihiro Kakeji[*1], Rintaro Yoshida[*1], Keisuke Ikeda[*1], Kojiro Nishida[*1], Tadashi Koga[*1], Akinori Egashira[*1], Eriko Tokunaga[*1], Masaru Morita[*1], Hideo Baba[*2] and Yoshihiko Maehara[*1] (*[1]*Dept. of Surgery and Science, Graduate School of Medical Sciences, Kyushu University, *[2]*Dept. of Gastroenterological Surgery, Graduate School of Medical Sciences, Kumamoto University*)

Summary

We are conducting a prospective randomized trial to evaluate the survival benefit of adjuvant chemotherapy with S-1 (tegafur, gimeracil, oteracil potassium) and UFT (uracil-tegafur) after curative surgery for patients with Stage II and III rectal cancer. Patients are randomized to either administration of UFT (control) or S-1. UFT was orally administered for 5 days (400 mg/m²/day) followed by two days rest for a year. S-1 was orally administered for 4 weeks (80 mg/m²/day) followed by two weeks rest for a year. The primary endpoint is relapse-free survival (RFS) rate, and the secondary endpoints are overall survival time (OS) and frequency or level of adverse events. We aim to include 400 patients in each of the treatment groups and assume that the registration period will last until 2009. Key words: UFT, S-1, Randomized trial, Rectal cancer, Adjuvant chemotherapy, Corresponding author: Eiji Oki, Department of Surgery and Science, Graduate School of Medical Sciences, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan

要旨　治癒切除を受けたStage IIおよびStage III（TNM分類）の直腸癌（Rsを除く）症例を対象として，術後補助化学療法としてのS-1（tegafur・gimeracil・oteracil potassium）療法の有用性を証明するため，UFT（tegafur・uracil）療法を対照としてランダム化比較試験を開始した。primary endpointは無再発生存期間でsecondary endpointは生存期間と有害事象の程度と頻度とした。UFTは400 mg/m²/day（tegafur相当量）を1日2回に分けて5日間連日経口投与し，その後2日間休薬する。これを1年間継続する。S-1は80 mg/m²/day（tegafur相当量）を1日2回に分けて4週間連日経口投与し，その後2週間休薬，これを1年間継続するスケジュールである。予定症例数は各治療群400例，登録期間は2009年までで，最終登録より5年間を観察期間としている。

[*2] 熊本大学大学院医歯薬研究院・消化器外科

連絡先: 〒812-8582 福岡市東区馬出3-1-1　[*1] 九州大学大学院医学研究院・消化器・総合外科
沖　英次

0385-0684/06/¥500/論文/JCLS

I. 背 景

固形腫瘍の治療においては，外科的切除が第一選択とされている。その治療成績の向上を図る目的で種々の術後補助化学療法が検討されている。欧米とは手術手技が異なり，日本では術後補助化学療法に経口フッ化ピリミジン系抗癌剤が主に用いられていた。しかしエビデンスという観点からは問題点が指摘されていた。近年，経口フッ化ピリミジン系抗癌剤であるUFTにおいて，肺癌[1]，直腸癌[2]，乳癌[3]および胃癌[4]の領域で術後補助化学療法としての有用性が報告された。一方，1999年に承認となったS-1は経口フッ化ピリミジン系抗癌剤として，効果増強と消化管での副作用軽減を目的に開発された薬剤で，胃癌で46.5%という高い奏効率を有し，早期に承認となった薬剤である。今回，直腸癌の術後補助化学療法の成績向上を目的として，UFTをコントロールとしS-1の有用性を検討することを計画した。

II. 対象症例および投与法

組織学的に直腸(Rsを除く)の腺癌と診断され，Table 1を満たす症例に対して以下の治療をFAXによる中央登録方式で無作為に選択して行う。

1．対照群（A群）

手術+UFT: UFTは400 mg/m²/day（tegafur相当量）を1日2回に分けて経口投与する。これを5日間連日経口投与し，その後2日間休薬するスケジュール(5投2休法）で1年間投与する。

2．試験群（B群）

手術+S-1: S-1は80 mg/m²/day (tegafur相当量）を1日2回に分けて経口投与する。これを4週間連日経口投与し，その後2週間休薬するスケジュールで1年間投与する（Fig. 1)。

III. 試験デザインの解説

本試験の目的は，治癒切除を受けたStage IIおよびStage III（TNM分類）の直腸癌（Rsを除く）症例を対象として，術後補助化学療法としてのS-1（tegafur・gimeracil・oteracil potassium）療法の有用性をUFT (tegafur・uracil）療法を対照としてランダム化比較試験にて検証することである。primary endpointは無再発生存期間 (relapse-free survival: RFS) であり，secondary endpointは生存期間 (overall survival: OS) である。

S-1はUFTを上回る効果が期待できる反面，副作用に関してもUFTより高頻度で発現することが予想される。また，どちらの薬剤も経口剤であり外来治療であるという点を含め，利便性に関しては同等と考えられる。したがって，S-1療法はprimary endpointであるRFSにおいて，UFT療法に勝る結果がでて初めて本試験終了後の標準的治療の一つと位置付けられる。このためS-1療法がUFT療法に比してRFSの延長効果があるか否かの優越性を検証することとした。

本試験の主たる仮説は「試験群（S-1療法）のRFSが

Table 1 Eligibility criteria

1) Histologically confirmed rectal cancer except Rs
2) Histological Stage II A (T 3, N 0, M 0), II B (T 4, N 0, M 0), III A (T 1-2, N 1, M 0), III B (T 3-4, N 1, M 0), and III C (any T, N 1, M 0) located in rectal except Rs (according to TMN classified, UICC 6th edition, 2002)
3) Patients without synchronous multiple colorectal carcinoma deeper than histological depth of invasion, sm
   Synchronous colorectal carcinoma with histological tumor invasion of mucosa is permitted, if patients have underwent curative resection.
4) Curative resection with D 2 or more lymph node dissection
5) Resection of histological curability A was performed
6) Age≦20 and≧80 years
7) No prior treatment (chemotherapy and radiotherapy etc) except for resection
8) Adjuvant chemotherapy will be started within 7 weeks after the operation
9) Oral administration of UFT or S-1 is possible
10) Sufficient organ functions drawn within 14 days of entry
   a) WBC≧3,500/mm³
   b) Hemoglobin≧9.0 g/dl
   c) Platelet≧100,000/mm³
   d) Total bilirubin≦1.5 mg/dl
   e) AST, ALT<100 IU/l
   f) Serum creatinine≦1.0 mg/dl

Written informed consent is required

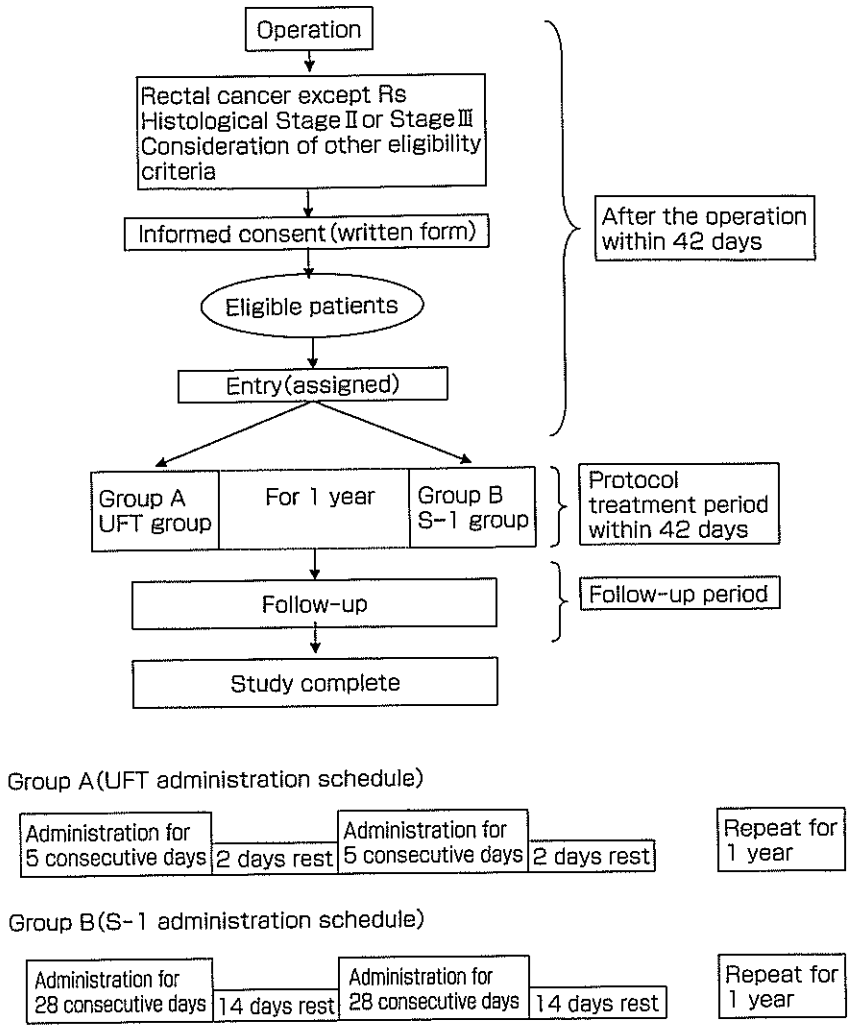

Fig. 1 Flow chart of the registration of the study
Patients were randomized either to Group A (UFT group) or Group B (S-1 group).
Group A: UFT was administered orally for 5 days (400 mg/m²/day) followed by two days rest for a year.
Group B: S-1 was orally administered for 4 weeks (80 mg/m²/day) followed by two weeks rest for a year.

対照群（UFT 療法）に対して有意に上回った場合，S-1 療法をより有用な治療法と判断する」としている。これまでの報告によると UFT 療法の直腸癌術後補助化学療法の成績では，TAC-CR において 5 年無再発生存率が 73.6%，大腸癌術後補助化学療法研究委員会: National Surgical Adjuvant Study of Colorectal Cancer（N・SAS-CC）では 3 年無再発生存率が 78%であった。

本試験での primary endpoint の解析の中心は死亡・再発をイベントに含む 5 年無再発生存率であり，N・SAS-CC では 3 年無再発生存率であるため，上記二つの結果より下回ることが予想されるために UFT 群の 5 年無再発生存率を 70%と仮定した。一方，S-1 群の予後改善効果を臨床的に意味があると思われるハザード比 0.7 と仮定した。したがって登録 3 年，追跡 5 年，α=5%（両側），検出力 80%として，S-1 群の優越性の検証に必要な症例数を算出すると 1 群当たり 381 例，両群 762 例となる。

IV. 考　察

1．欧米における直腸癌の術後補助化学療法

直腸癌について欧米では全直腸間膜切除（total mesorectal excision）の普及をみるのみで，直腸間膜外リンパ節転移（側方転移）に対する外科治療は試みられていない。その理由として，1950 年代の Stearns ら[5]，Bacon ら[6]の側方骨盤リンパ節郭清の試みが否定的な結果であったこと，側方転移は外科治療の対象ではなく全身病の一部ととらえ，放射線化学療法の対象と考えられていることおよび動脈硬化が高度である欧米人にとって側方郭清の侵襲と機能障害は高度であることなどがあげられ，成績向上に対する欧米の戦略は術前，術後の放射

線治療を含む補助療法に向けられてきた。

Stage IIおよびStage IIIの直腸癌に対する術後放射線照射および5-FUをベースとする化学療法の効果は，Gastrointestinal Tumor Study Group (GITSG) 7175[7]，Mayo/North Center Cancer Treatment Group (NCCTG) 79-47-51[8]およびNational Surgical Adjuvant Breast and Bowel Project (NSABP) R-01[9]によって確立された。こうした研究から，外科切除後に放射線療法と化学療法とを併用すれば，無病期間および全生存とも増大することが明らかになった。また，この試験成績の発表後，National Cancer Institute (NCI)（米国国立癌研究所）は，1990年のConsensus Development Conference（コンセンサス開発会議）で，Stage IIおよびStage IIIの直腸癌患者に対して術後併用療法による治療を推奨するという結論に達した。

これに引き続いて，GITSG[10]，Intergroup 86-47-51試験[11]，Intergroup試験0144[12]，SWOG-9304[13]，NSABP R-02[14]が実施され，NSABP R-02によってAmerican Society of Clinical Oncology (ASCO)では術後放射線療法の果たすべき役割に関する議論が始まった。National Cancer Data Baseに登録された5,987人のStage IIIの解析の結果では，5年生存率は手術単独でStage IIIA 39.0%，Stage IIIB 21.7%，Stage IIIC 12.2%，補助化学放射線療法を実施しても，Stage IIIA 60.0%，Stage IIIB 40.9%，Stage IIIC 28.9%（American Joint Committee on Cancer Sixth edition）と，日本に比べてその予後は悪い[15]。なお，2006年のNational Comprehensive Cancer Networkのガイドライン[16]によると，Stage IIおよびStage III症例には術前に5-FU+放射線治療を行った場合は術後に5-FU±Leucovorin（もしくはFOLFOX）を使用し，術前放射線療法を行わなかった場合は，術後に5-FU±Leucovorin（もしくはFOLFOX）次いでcontinuous 5-FU/放射線療法にさらに5-FU±Leucovorin（もしくはFOLFOX）と，結腸癌における術後補助化学療法のエビデンスも取り入れたものとなっている。ただしFOLFOXはあくまで結腸癌でのエビデンスであり，直腸癌にはエビデンスはない。

2．日本における直腸癌の術後補助化学療法

日本では骨盤内自律神経を温存しつつ側方郭清を行う骨盤自律神経温存側方骨盤リンパ節郭清（自律神経温存D3郭清術）が普及している。全直腸間膜切除を含んだ手術が一般に行われている欧米に対して手術成績が良好で，局所再発率が低いことを理由に，術後放射線治療あるいは化学放射線治療は十分に検討されていない。しかしながら，日本で普及されている側方郭清を伴う自律神経温存D3郭清術と，欧米での標準手術である直腸間膜切除（mesorectal excision）での比較試験は行われておらず，そのため現在これらの術式の有効性および手術に関連する合併症の発生率などを検討するために，ランダム化比較試験が実施されている[17]。

術後補助化学療法については，1970年代より結腸癌と直腸癌を対象として手術単独に対する比較試験が行われてきた。その結果，結腸癌では術後補助化学療法の有用性を示した試験はほとんど皆無であったのに対し，直腸癌においては，Dukes BおよびCを対象とした四つの試験[18-21]により術後補助化学療法の有用性が示唆された。

しかしこれまでの国内の臨床試験では試験デザイン・運営管理が不十分であること，転移/再発例では有効性が認められていない少量投与法が採用されていたこと，多施設少数症例登録であり施設間差が大きいことなどから，術後補助化学療法群が手術単独群と比較して明確に有用であるという結果は得られていない。そこで，これらの術後補助化学療法の有用性を検討する目的で，1996年よりDukes C大腸癌を対象とした手術単独群とUFT投与群（400 mg/m² 5日投与2日休薬1年間）との比較試験が，N・SAS-CCにて行われた。その結果，直腸癌においては中間解析の段階で3年無再発生存率が手術単独群60%，UFT投与群78%（p=0.0014），3年生存率が手術単独群81%，UFT投与群91%（p=0.0048）と明らかに予後を改善することが判明し，早期公表すべきとの答申がなされて2004年のASCOにて報告された[2]。これらのことより，UFTはStage III直腸癌の治癒切除例に対する術後補助化学療法として，明らかなエビデンスを示している。さらに，これまで施行されたDukes B (Stage II)，Dukes C (Stage III)の直腸癌に対してUFTを使用した五つの無作為化比較試験（集学・特7[20]，集学・特15-1[22]，集学・特15-2[22]，TAC-CR[21]，N・SAS-CC[2]）のmeta-analysisでも，5年無再発生存率が手術単独群57.9%，UFT群67.6%と，UFT群が有意に無再発生存率を改善させたとの報告が2005年のASCOにて報告されている[23]。

3．本試験設定の意義

直腸癌については，欧米と日本とで放射線療法の有無と手術内容，予後に差がある。一部では，進行直腸癌の欧米での標準治療は，すでに術後にFOLFOXを行うことが標準であると考えられがちであるが，実際は上記のように放射線治療を含むレジメが術前もしくは術後に必ず導入され，術後に5-FU±LeucovorinやFOLFOX単独治療を行うことに対する標準治療としてのエビデンスはない。日本と欧米ではリンパ節の郭清方法に関する考え方に大きな違いがあり，直腸癌に関しては，欧米での