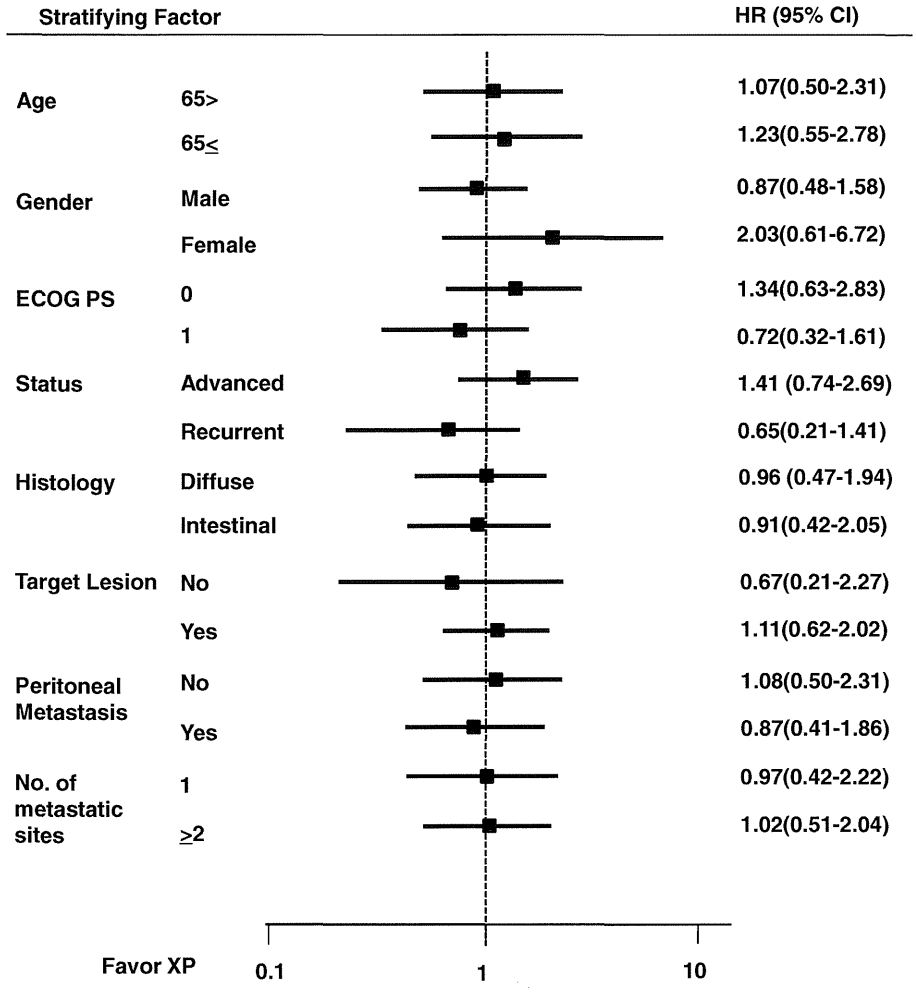

Fig. 3 Subset analysis of overall survival under SP and XP treatment regimens. This subset analysis suggests no apparent interaction between the treatment effect of each treatment and patient characteristics

chemotherapy (mainly taxanes or irinotecan), and 28 patients (56 %) in the SP group and 18 patients (69 %) in the XP group received third-line chemotherapy, with no statistically significant differences between groups.

Toxicity

Toxicity is shown in Table 3. The frequency of any grade 3–4 hematological toxicity was 42 % (21 of 50 patients) in the SP group and 35 % (9 of 26 patients) in the XP group, with no significant difference ($p = 0.53$). Neutropenia was the most common grade 3–4 hematological toxicity in both treatment groups (34 % in SP and 27 % in XP; $p = 0.53$). The frequencies of any grade 3–4 non-hematological toxicity were also not different between the SP and XP groups (30 vs. 19 %; $p = 0.31$). Although grade 1 or 2 hand–foot syndrome was significantly more common in the XP group than in the SP group (46 vs. 8 %; $p < 0.01$), no patients experienced hand–foot syndrome of grade 3 or more (grade 1 in 7 patients and grade 2 in 5 patients in the XP group; grade 1 in 2 patients and grade 2 in 2 patients in the SP group).

Discussion

In this study, we retrospectively compared the efficacy of first-line chemotherapy with SP or XP in AGC patients. Our results indicated that SP and XP showed very similar efficacy in terms of response rates, PFS, and OS. There was also no significant difference in toxicity between treatments other than mild hand–foot syndrome. These treatment results suggested that either treatment can be considered a first-line treatment option for patients with AGC in Japan.

As described before, in phase II studies, S-1 showed a high response rate (>40 %) even as monotherapy [7, 8]. Additionally, S-1 combined with cisplatin, another key drug for treatment of AGC, showed superior efficacy to S-1 alone [4] and has now became the standard chemotherapy for AGC in Japan. However, in a large, non-Japanese, phase III trial (the First-Line Advanced Gastric Cancer Study; FLAGS trial), SP did not show superiority compared with 5-FU plus cisplatin, although exploratory analysis demonstrated significant non-inferiority with

Table 3 Toxicities

Toxicities	SP ($n = 50$)		XP ($n = 26$)		p value*
	All (%)	Grade 3–4 (%)	All (%)	Grade 3–4 (%)	
Hematological toxicity					
Any	35 (70)	21 (42)	19 (73)	9 (35)	0.53
Leukopenia	25 (50)	3 (6)	17 (65)	1 (4)	0.69
Neutropenia	30 (60)	17 (34)	19 (73)	7 (27)	0.53
Anemia	32 (64)	7 (14)	14 (54)	5 (19)	0.55
Thrombocytopenia	11 (22)	1 (2)	7 (27)	0	0.48
Non-hematological toxicity					
Any	41 (82)	15 (30)	19 (73)	5 (19)	0.31
Nausea	30 (60)	7 (14)	12 (46)	3 (12)	0.76
Vomiting	8 (16)	1 (2)	5 (19)	1 (4)	0.63
Anorexia	32 (64)	10 (20)	14 (54)	4 (15)	0.62
Fatigue	22 (44)	2 (4)	9 (35)	2 (8)	0.49
Diarrhea	14 (28)	4 (8)	5 (19)	1 (4)	0.48
Increased creatinine	8 (16)	0	7 (27)	0	0.13**
Stomatitis	11 (22)	2 (4)	4 (15)	0	0.3
Rash	5 (10)	0	1 (4)	0	0.35**
Hand–foot syndrome	4 (8)	0	12 (46)	0	≤0.01**
Febrile neutropenia	2 (4)	2 (4)	0	0	0.3

* Comparison of grades 3 or more

** Comparison of all grades

fewer toxic effects [6]. The SP regimen in the FLAGS trial was different from that of the SPIRITS trial, with a lower dose of S-1 (50 mg/m^2) and a higher dose of cisplatin 75 mg/m^2 every 4 weeks. This regimen was based on a previous phase I study [14] which suggested that S-1 was less tolerable in Western patients than in Japanese patients. Although polymorphisms in CYP2A6 to convert the prodrug tegafur to 5-FU is one possible explanation regarding the differences in tolerability of S-1 between Western and Eastern populations [15, 16], the exact mechanism is still unknown. This difference in tolerability may be a drawback of SP when used as a reference arm in a global study.

A previous phase II study of capecitabine monotherapy in Japan showed an overall response rate of 23 % [17], which seemed to be lower than that of S-1 [7, 8]. However, that study used a lower dose of capecitabine (828 mg/m^2 twice daily for 3 and 1 week rest) than that used in other trials [12, 13, 18]. Lee et al. [18] performed a randomized phase II study of monotherapy of S-1 (40–60 mg/m^2 twice daily for 4 and 2 weeks rest) and capecitabine (1250 mg/m^2 twice daily for 2 and 1 week rest) for elderly AGC patients, and reported similar efficacies and safety for S-1 and capecitabine. Therefore, the low dose of capecitabine may have partially contributed to the lower response rates in the previous phase II study in Japan [17]. In the ToGA and AVAGAST studies [12, 13], capecitabine of 1000 mg/m^2 twice daily was combined with 80 mg/m^2 cisplatin, higher than the dose of cisplatin in the SP regimen with a shorter interval. Although in about half of the patients in our analysis the dose of cisplatin was reduced according to the defined protocol in each trial, the observed toxicity in the XP group was very similar to that in the SP group other than mild hand–foot syndrome. As described, four patients decreased dose of capecitabine due to hand–foot syndrome, but most patients could continue XP until PD after dose reduction. Grade 1 or 2 hand–foot syndrome was generally manageable with topical ointments or adequate dose reduction. Therefore, the XP regimen is considered tolerable in Japanese patients, although modification for toxicities may be important.

Importantly, these 2 types of fluoropyrimidine show some different characteristics in the mechanism of their antitumor effect. A subset analysis of the FLAGS trial showed that S-1 seemed to be better than 5-FU in diffuse-type gastric cancer [6]. This result was consistent with the results of a subset analysis of JCOG9912, which showed that S-1 was better than 5-FU in patients with diffuse-type gastric cancer or with gastric cancer associated with high dihydropyrimidine dehydrogenase (DPD), with diffuse-type tumors associated more commonly than intestinal type with high DPD [19]. This result was expected, since S-1 consists of tegafur, otastat potassium, and gimestat, which is a potent competitive inhibitor of DPD. Capecitabine is transformed to 5-FU in several steps, the last of which is conversion of 5′-deoxy-5-fluorouridine to FU by thymidine phosphorylase (TP). Capecitabine was designed to take advantage of the increased levels of TP observed in tumors in comparison with normal tissues, and is expected to

selectively exert an antitumor effect. Expression of TP is reported to be negatively associated with efficacy of 5-FU or S-1 in gastric cancer [20, 21] and colorectal cancer [22]. Capecitabine or 5′-deoxy-5-fluorouridine (a prodrug of capecitabine) has previously been reported to be effective in high TP gastric cancer [23–25]. In the aforementioned phase II trial [17], the response rate was significantly higher (Fisher's exact test, $p = 0.028$) in patients with TP-positive and DPD-negative tumors (60 %, 6/10) than in the remaining patients (13 %, 2/15). High TP expression in colorectal cancer is reported to be associated with higher efficacy of capecitabine combination therapy [26]. Therefore, the biomarkers TP and DPD may be candidates to select whether S-1 or capecitabine be used for each patient, although validation in a randomized study is necessary.

It is important to note the limitations of the present study. First, it was a retrospective non-randomized comparison. Although patients in the XP group had been treated in clinical trials, patients in the SP group had not been included in clinical trials for various reasons, so hidden selection bias may affect our comparison. Nevertheless, because our results for the SP group were quite similar to those of the SPIRITS trial in terms of efficacy and toxicity, the possibility of these biases may not be high. We also included patients with a history of other cancers and those who had had a short duration of S-1 chemotherapy in another hospital, although these patients are generally ineligible in clinical trials of first-line chemotherapy. However, the treatment results of the SP group in which these patients were excluded were almost the same as the treatment results of the all-patients cohort (data not shown). Second, patients in the XP group generally underwent response evaluation every 6 weeks as defined in the protocol. In contrast, patients in the SP group underwent response evaluation at various intervals: for example, every 5 (1 cycle of SP) or 10 weeks (2 cycles of SP). Therefore, results of PFS may not be comparable due to different timing of evaluation, although OS was not subject to this bias. Third, HER2 status was not evaluated in all patients, and the prognostic impact of HER2 remains unknown in this setting. Third, the small sample size from a single center study is another limitation.

In conclusion, although the retrospective nature of the study and the small number of patients is a major limitation, the SP and XP treatment regimens were associated with similar efficacy and safety for patients with AGC. To confirm our preliminary results, a randomized study of XP versus SP for AGC is ongoing in Japan, focusing especially on translational research (UMIN-ID:UMIN000006045, ClinicalTrials.gov-ID:NCT01406249).

Conflict of interest None of the authors have financial or personal conflicts of interest to disclose.

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ORIGINAL ARTICLE

Prognosis of patients with advanced gastric cancer by HER2 status and trastuzumab treatment

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Abstract
Background The purpose of this study was to evaluate the impact of human epidermal growth factor receptor 2 (HER2) status and trastuzumab treatment on the prognosis of patients with advanced gastric cancer (AGC).
Methods We retrospectively analyzed 364 AGC patients who received systemic chemotherapy. To evaluate the impact of trastuzumab exposure during any type of chemotherapy, our analysis used time-varying covariates to avoid a possible lead-time bias.
Results Among the 364 patients, 58 (15.9 %) were HER2-positive. The median overall survival of the HER2-positive patients treated with trastuzumab ($n = 43$) was significantly longer than that of the HER2-negative patients [$n = 306$; 24.7 vs. 13.9 months, with an adjusted hazard ratio (HR) of 0.58; 95 % confidence interval (CI), 0.36–0.95; $P = 0.03$]. Notably, 22 patients continued with trastuzumab beyond the date of progression. By contrast, the HER2-positive patients not treated with trastuzumab ($n = 15$) showed survival similar to that of the HER2-negative patients (13.5 vs. 13.9 months, with an adjusted HR of 1.04; 95 % CI, 0.52–2.11; $P = 0.91$). According to the multivariate analysis, exposure to trastuzumab was independently associated with a better prognosis (HR 0.56; 95 % CI; 0.33–0.93; $P = 0.026$).
Conclusions Recent HER2-positive AGC patients have a better prognosis than HER2-negative patients, particularly when treated with trastuzumab.

Keywords Chemotherapy · Gastric cancer · HER2 · Trastuzumab

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Introduction

Gastric cancer is the fourth most common malignancy in the world (988,602 cases in 2008, 7.8 % of the total cancer cases) and the second leading cause of cancer deaths (737,419 deaths, 9.7 % of the total) [1]. Although the most effective treatment for localized disease is surgery, approximately half of all patients with advanced-stage disease experience a recurrence following a curative resection. The prognosis of patients with advanced or recurrent gastric cancer (AGC) remains poor, and commonly used combination chemotherapy regimens, consisting of a fluoropyrimidine plus a platinum agent with or without docetaxel or anthracyclines, lead to a median overall survival

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(OS) of only 1 year [2–7]. Therefore, the development of novel anticancer agents or strategies for treating AGC is urgently required.

Human epidermal growth factor receptor 2 (HER2) is a growth factor receptor that is overexpressed in approximately 10–30 % of gastric cancers [8, 9]. The main function of HER2 is to mediate cell growth, differentiation, and survival [10, 11]. Therefore, HER2-positive tumors are expected to have more aggressive characteristics than HER2-negative tumors. Numerous studies have shown that HER2-positive breast cancer is associated with a poor prognosis when compared with HER2-negative breast cancer [11, 12]. Similarly, the prognostic value of HER2 status in gastric cancer has been evaluated in a number of reports. Although recent studies of gastric cancer have shown no impact of HER2 on survival [13, 14], the majority of the past studies have suggested that HER2-positive gastric cancer was associated with a poorer prognosis than HER2-negative gastric cancer [9, 15, 16].

Trastuzumab, a humanized monoclonal antibody that targets HER2, has recently been shown to improve the prognosis of HER2-positive AGC [17]. Combination chemotherapy consisting of 5-fluorouracil (5-FU) or capecitabine plus cisplatin with trastuzumab has shown a significantly higher response rate (47 vs. 35 %, $P = 0.0017$), a longer progression-free survival [PFS; 6.7 vs. 5.5 months; hazard ratio (HR) = 0.71, 95 % confidence interval (CI) 0.59–0.86], and a longer OS (13.8 vs. 11.1 months; HR = 0.74, 95 % CI 0.60–0.91) than chemotherapy alone [17]. This difference was more prominent in patients with immunohistochemical staining (IHC) of 3 + (IHC 3 +) or IHC 2 + plus positive gene amplification according to fluorescence in situ hybridization (FISH) (16.0 vs. 11.8 months; HR = 0.65, 95 % CI 0.51–0.83). The risk reduction achieved with trastuzumab is almost comparable to that found in a pivotal study of breast cancer [18]. According to these results, chemotherapy with trastuzumab is considered to be standard chemotherapy for HER2-positive AGC. However, the question of whether trastuzumab improves the prognosis of HER2-positive AGC patients to the levels observed in HER2-negative disease still remains. HER2-positive breast cancer patients who receive trastuzumab have been reported to have a better prognosis (HR of 0.56) than women with HER2-negative breast cancer [19].

The purpose of this study was to evaluate the impact of HER2 status and trastuzumab treatment on the prognosis of patients with AGC. We evaluated the impact of trastuzumab on OS using a time-varying covariate (TVC) analysis.

Patients and methods

Patients

This retrospective study was designed to compare the OS of HER2-positive and HER2-negative AGC patients who received systemic chemotherapy. In this analysis, HER2 positivity was defined as IHC 3 + or IHC 2 + plus FISH positivity, because these criteria were considered to be indications for using trastuzumab by a subset analysis of the ToGA trial [17, 20]. The following additional principal inclusion criteria were used: the presence of histologically proven, inoperable gastric cancer in patients who had received systemic chemotherapy; an Eastern Cooperative Oncology Group performance status (ECOG PS) of 0–2; and sufficient bone marrow, liver, and renal function.

Between April 2005 and August 2011, 807 consecutive patients with AGC received systemic chemotherapy at our institution, 650 of whom met the inclusion criteria. Among those 650 patients, 364 patients were evaluated for HER2 to screen them for the ToGA study or for trastuzumab treatment, and these 364 patients were analyzed in this study. Although trastuzumab had not yet been approved in Japan at the time of our study, using trastuzumab for HER2-positive patients was approved by our institution based on the results of the ToGA study. We note that fluoropyrimidine (5-FU or the oral fluoropyrimidine S-1), cisplatin, docetaxel, and paclitaxel were approved for use and were consistently available throughout this period. Written informed consent for the chemotherapy was obtained from all of the patients.

Statistical analysis

We compared the OS according to HER2 status and trastuzumab exposure by dividing the patients into three groups (HER2-negative vs. HER2-positive treated with trastuzumab vs. HER2-positive treated without trastuzumab). The OS was estimated from the date of systemic chemotherapy initiation to the date of death or last known date of survival. The vital status and disease status were confirmed by checking the medical records from the date of the last follow-up visit. In the cases that were lost to follow up, the vital status was confirmed by the census record, which is conducted annually at our institution. The median OS rate was estimated by the Kaplan–Meier method. The baseline patient characteristics and exposure to each class of chemotherapeutic agent, fluoropyrimidine (5-FU or oral fluoropyrimidine), platinum agents (cisplatin or oxaliplatin), taxanes (docetaxel or paclitaxel), and irinotecan and trastuzumab, were evaluated. Because other agents (mitomycin C or methotrexate) were also commonly used as

salvage therapy at our institution, we also evaluated exposure to these treatments. The disease progression associated with each line of chemotherapy was measured from the beginning of treatment to the date of disease progression, as evaluated by the attending physician.

The differences in the OS of each group were evaluated by univariate and multivariate analyses using a Cox proportional hazards model and presented as an HR and a 95 % CI. A subset analysis was stratified by the characteristics of the patients with significant differences in the three groups. We also evaluated the association between the exposure to each class of chemotherapeutic agent and the OS using multivariate analyses, because differences in exposure to agents other than trastuzumab may have contributed to survival differences. Because the length of exposure to each agent class varied over time (i.e., between first-, second-, and third-line treatments), the analyses may have been compromised by lead-time bias, which would result in false-positive or false-negative associations between a larger number of chemotherapeutic lines and longer survival. To minimize this potential bias, the exposure to each agent class was analyzed as a TVC. In addition, because disease progression was the primary reason for proceeding to the next line of chemotherapy, tumor progression during each line of chemotherapy was included in the TVCs. Each TVC was constructed as a step function initially set at 0 and increased by 1 U each time the corresponding event was observed. This method has been used in several reports that have evaluated the impact of drug exposure on survival [21–23].

Other variables considered in the multivariate analyses were ECOG PS (0 vs. $\geq$1), gender, histological type (diffuse vs. intestinal), age (< 65 vs. $\geq$65 years), previous gastrectomy (no vs. yes), disease status (advanced vs. recurrent), prior adjuvant chemotherapy (no vs. yes), presence of liver metastasis (no vs. yes), presence of peritoneal metastasis (no vs. yes), number of metastatic sites (1 vs. $\geq$2), and first-line chemotherapy (monotherapy vs. combinations). The *P* values for testing differences in the baseline characteristics of each group were calculated with the χ^2 test for homogeneity or trend or with Fisher's exact test.

The statistical analyses were performed using STATA ver. 10 (Stata Corp LP, College Station, TX, USA). All of the tests were two-sided, and *P* values less than 0.05 were considered to be statistically significant.

Results

Patient characteristics

The patient characteristics according to HER2 status and trastuzumab use are shown in Table 1. Among the HER2-evaluated patients, 58 (15.9 %) were HER2-positive. HER2-positivity was more frequently associated with intestinal-type histology, liver metastasis, and lymph node metastasis. By contrast, peritoneal metastasis was more common in the HER2-negative patients than in the HER2-positive patients (Table 1). Among the HER2-positive patients, 43 received trastuzumab; 23 patients started it as first-line therapy (9 of these patients were included in the ToGA study) and 20 started it as second- or further-line therapy. Among the patients who were treated with trastuzumab who were not in the ToGA study, 22 patients continued with trastuzumab beyond the date of progression. The median duration of trastuzumab treatment in the 43 patients receiving this agent was 8.4 months (range 0.5 to 25 +), and 28 patients were still continuing with trastuzumab treatment at the time of this analysis. The frequency of exposure to platinum agents tended to be higher in HER2-positive disease, although no other significant differences were observed between the 3 groups (Table 1).

Survival and multivariate analysis

The median follow up at the time of the analysis was 38.9 months. The median OS of the HER2-positive patients ($n = 58$; 24.1 months; 95 % CI, 14.9–31.1 months) was significantly longer than that of the HER2-negative patients ($n = 304$; 13.9 months; 95 % CI, 12.7–16.1 months) by univariate analysis (HR 0.68; 95 % CI, 0.46–0.99; $P = 0.045$), although the difference was not significant in the multivariate analysis (HR 0.67; 95 % CI, 0.44–1.02; $P = 0.067$). The median OS of the HER2-positive patients treated with trastuzumab ($n = 43$; 24.7 months; 95 % CI, 15.6–35.1 months) was significantly longer than that of the HER2-negative patients (13.9 months), as verified by univariate analysis (HR 0.57; 95 % CI, 0.36–0.90; $P = 0.015$, Fig. 1) and multivariate analysis (adjusted HR of 0.58; 95 % CI, 0.36–0.95; $P = 0.03$. By contrast, the HER2-positive patients not treated with trastuzumab ($n = 15$) showed survival similar to that of the HER2-negative patients (13.5 months; 95 % CI, 3.6 to not reached), as demonstrated by univariate analysis (HR 1.11; 95 % CI, 0.58–2.08; $P = 0.76$) and multivariate analysis (HR 1.04; 95 % CI, 0.52–2.11; $P = 0.91$). The subset analysis according to the characteristics of selected patients demonstrated consistently higher rates of survival among HER2-positive patients treated with trastuzumab than among HER2-negative patients, with no significant heterogeneity (Table 2).

According to the multivariate TVC analysis, exposure to trastuzumab was independently associated with an improved prognosis (HR 0.56, 95 % CI, 0.33–0.93; $P = 0.026$, Table 3). Additionally, exposure to fluoropyrimidine, taxanes, and irinotecan was associated with a better prognosis, and exposure to platinum agents was

Table 1 Patient characteristics

Characteristics	HER2-positive patients treated with trastuzumab ($n = 43$, %)	HER2-positive patients not treated with trastuzumab ($n = 15$, %)	HER2-negative patients ($n = 306$, %)	*P* value
Age (years)				
Median (range)	63 (30–78)	68 (45–80)	64 (30–93)	0.33
Gender				0.45
Male	28 (65)	12 (80)	196 (64)	
Female	15 (35)	3 (20)	110 (36)	
ECOG PS				0.52
0	23 (53)	7 (47)	123 (40)	
1	17 (40)	6 (40)	149 (49)	
2	3 (7)	2 (13)	34 (11)	
Histological type				<0.001
Diffuse	12 (28)	4 (27)	217 (71)	
Intestinal	31 (72)	11 (73)	99 (29)	
Site of primary tumor				<0.001
EG junction	8 (19)	5 (33)	28 (9)	
Gastric	35 (81)	10 (67)	278 (91)	
Disease status				0.49
Advanced	33 (77)	9 (60)	203 (66)	
Recurrent	10 (23)	6 (40)	103 (34)	
Previous gastrectomy				0.59
No	26 (60)	8 (53)	160 (52)	
Yes	17 (40)	7 (47)	146 (48)	
Adjuvant chemotherapy				0.81
No	34 (79)	13 (87)	248 (81)	
Yes	9 (21)	2 (13)	58 (19)	
Site of metastasis				
Lymph node	27 (63)	12 (80)	145 (47)	0.01
Peritoneum	12 (28)	5 (33)	172 (56)	0.001
Liver	21 (49)	6 (40)	71 (23)	0.001
Number of metastatic organs				0.11
1	15 (35)	8 (53)	174 (57)	
2 or more	28 (65)	7 (47)	132 (43)	
First-line CTx				0.03
Combination	35 (81)	12 (80)	192 (63)	
Monotherapy	8 (19)	3 (20)	114 (37)	
Initiation of CTx				0.1
2005–2007	10 (23)	8 (53)	96 (31)	
2008–2011	33 (77)	7 (47)	210 (69)	
Exposure to agents				
Fluoropyrimidine	38 (88)	13 (87)	292 (95)	0.08
Platinum	42 (98)	11 (73)	228 (75)	0.003
Taxane	33 (77)	12 (80)	206 (67)	0.29
Irinotecan	22 (51)	6 (40)	137 (45)	0.67
Others	5 (12)	1 (7)	49 (16)	0.49

Underlined *P* values are significant

HER2 human epidermal growth factor receptor 2, *PS* performance status, *ECOG* Eastern Cooperative Oncology Group, *CTx* chemotherapy, *EG* esophagogastric

associated with improved survival, although the significance was borderline. By contrast, the other agents had no impact on survival.

Discussion

In this study, we compared the OS in AGC patients according to HER2 status and exposure to trastuzumab. The OS of the HER2-positive patients who were treated with trastuzumab was significantly longer than that of the HER2-negative patients, with an adjusted HR of 0.58. This result is similar to those observed in a large study of patients with breast cancer in which a similar degree of risk reduction was found [18]. In addition, the multivariate TVC analysis in our study showed that trastuzumab had a significant impact on OS. These results confirmed that trastuzumab therapy improves the prognosis of HER2-positive AGC patients beyond that of patients with HER2-negative AGC.

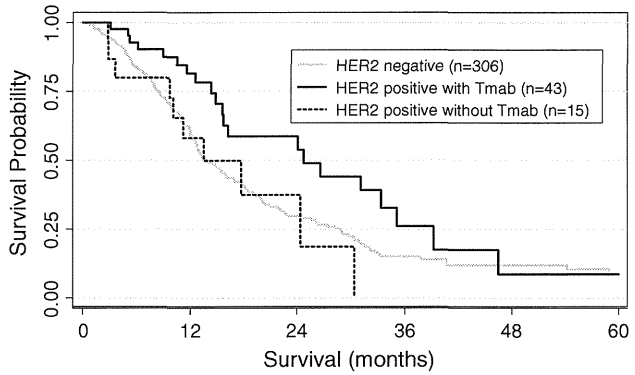

Fig. 1 The median OS of the HER2-positive patients treated with trastuzumab ($n = 43$; 24.7 months; 95 % CI, 15.6 to 35.1 months) was significantly longer than that of the HER2-negative patients $n = 306$; (13.9 months), as verified by univariate analysis (HR 0.57; 95 % CI, 0.36 to 0.90; $P = 0.015$) and multivariate analysis (adjusted HR of 0.58; 95 % CI, 0.36 to 0.95; $P = 0.03$). The HER2-positive patients not treated with trastuzumab ($n = 15$) showed survival similar to the HER2-negative patients (13.5 months; 95 % CI, 3.6 to not reached), as demonstrated by univariate analysis (HR 1.11; 95 % CI, 0.58 to 2.08; $P = 0.76$) and multivariate analysis (HR 1.04; 95 % CI, 0.52 to 2.11; $P = 0.91$)

In this study, the HER2-positive patients not treated with trastuzumab showed survival rates similar to those of the HER2-negative patients. By contrast, the majority of previous studies have found poorer survival among patients with HER2-positive gastric cancer than among patients with HER2-negative tumors [9, 15, 16]. Most of these studies have been based on retrospective analyses in a single institution that combined patients with advanced-stage cancer and those with resected gastric cancer. Additionally, until the ToGA study, various definitions were used to evaluate HER2 status. Recently, Janjigian et al. [14] reported the results of a retrospective analysis of HER2 status among 381 patients who had enrolled in several clinical AGC studies. The HER2-positive patients (IHC 3 + or FISH +; $n = 78$) tended to have a better prognosis than the HER2-negative patients (13.9 vs. 11.4 months), although a multivariate analysis showed no impact of HER2 status on survival [14]. Additionally, an exploratory analysis of HER2 status in the AVAGAST study showed similar median OS in the HER2-negative and HER2-positive patients (10.5 vs. 9.8 months) who received capecitabine or 5-FU plus cisplatin [24]. Combined with our results, these findings suggest that HER2 status may

Table 2 Impact of HER2 and trastuzumab use according to selected backgrounds

	HER2-negative	HER2-positive	
		Treated with trastuzumab HR (95 % CI)	Not treated with trastuzumab HR (95 % CI)
Histology			
Diffuse	1 (reference)	0.34 (0.12–0.99)	2.48 (0.67–8.90)
Intestinal	1 (reference)	0.53 (0.28–1.03)	0.90 (0.35–2.28)
p-heterogeneity		0.48	0.22
Site of metastasis			
Liver	1 (reference)	0.65 (0.28–1.51)	1.09 (0.24–4.74)
Lymph node	1 (reference)	0.68 (0.34–1.36)	0.78 (0.32–1.78)
Peritoneum	1 (reference)	0.76 (0.32–1.83)	1.00 (0.28–3.57)
p-heterogeneity		0.97	0.91
Initiation of chemotherapy			
Before 2008	1 (reference)	0.65 (0.27–1.59)	1.34 (0.56–3.24)
After 2008	1 (reference)	0.51 (0.25–1.06)	1.95 (0.25–15.27)
p-heterogeneity		0.69	0.87

Except for the stratifying factors, the HRs were adjusted for age, gender, ECOG PS, disease status, prior gastrectomy, adjuvant chemotherapy, and the number of metastatic sites

HR hazard ratio, *CI* confidence interval

Table 3 Results of the multivariate TVC analyses

Variable	Multivariate analysis with TVC[a]		
	HR	95 % CI	*P*
Exposure to agents			
Trastuzumab	0.56	0.33–0.93	0.026
Fluoropyrimidine	0.40	0.19–0.84	0.015
Platinum	0.63	0.38–1.03	0.06
Taxanes	0.37	0.25–0.57	<0.001
Irinotecan	0.47	0.33–0.67	<0.001
Other	0.85	0.55–1.38	0.39

Underlined *P* values are significant

TVC time-varying covariate, *HR* hazard ratio, *CI* confidence interval

[a] Adjusted by age, gender, ECOG PS, disease status, histology, prior gastrectomy, adjuvant chemotherapy, presence of liver metastasis, presence of peritoneal metastasis, number of metastatic sites, time of initiation of chemotherapy, and tumor progression during each line of chemotherapy

have a small impact on survival in AGC. In addition, recent data from a large randomized study of resected gastric cancer found that HER2 status was not associated with the OS or with relapse-free survival in either the group who received surgery only or the group who also received adjuvant chemotherapy [13].

Interestingly, the risk reduction or median survival with trastuzumab treatment in our analysis was relatively better than the results of a subset analysis among those patients with IHC 3 + or IHC2 + plus FISH + in the ToGA study [17]. Several possibilities may explain this difference. First, the median exposure to trastuzumab in the ToGA study was reported to be 4.9 months [17]. Although the reasons for discontinuing the treatment were not reported in detail, continuing with trastuzumab beyond progression was not allowed in the ToGA study. By contrast, the median duration of exposure to trastuzumab in our study was longer (8.4 months). A number of patients who were treated outside of the ToGA study continued with trastuzumab beyond progression because this strategy has been found to contribute to improved treatment outcomes in randomized studies of breast cancer patients [25, 26]. A prospective study that evaluates the importance of trastuzumab beyond progression is warranted, as is further evaluation of AGC patients in a randomized study. Second, owing to the retrospective nature of the present study, unblinded bias may have overestimated the treatment effect. Regardless, our results reconfirmed the results of the ToGA study, which demonstrated that a new effective agent with the right target improved the prognosis of gastric cancer. Additionally, other agents targeting HER2 (such as lapatinib, which has already been shown to be effective in HER2-positive breast cancer) are under investigation in AGC, and the results are awaited.

It is important to note the methodological limitations of the present study. First, not all of the patients in this study period were evaluated for their HER2 status. Thus, the analysis may have been subject to some selection bias, although no significant differences other than PS were observed between the patients in the analysis and the patients not evaluated for HER2 status during the same period (data not shown). The median OS of the patients not evaluated for HER2 was 13.0 months, which was similar to that of the HER2-negative patients or HER2-positive patients not treated with trastuzumab in this analysis. Second, the study was a retrospective, non-randomized comparison of trastuzumab treatment in patients evaluated for HER2 status. Therefore, the differing characteristics of the patients and the differing treatments (other than with trastuzumab) in each group may have affected the results. Because the use of trastuzumab and the exposure to platinum agents differed between the groups, we used the TVC analysis to adjust the exposure to agents and comprehensively evaluate the impact of each agent class, regardless of the treatment line. However, TVC analysis is not necessarily adequate under the conditions of our study because its validity may depend on the assumption of a strong association between treatment selection at the time of the events and the history leading up to the events [27]. Other potential confounders, such as PS and the metastatic site, were also considered in the multivariate analyses; owing to the retrospective, non-randomized nature of the study, however, residual confounding effects caused by non-included factors cannot be completely ruled out. Nevertheless, because our survival results for the HER2-negative patients and the HER2-positive patients not treated with trastuzumab were quite similar to those of the ToGA trial and the results of other randomized controlled trials, the effect of this selection bias may have been small. Third, the small sample size and single-center population are other major limitations of this study.

In conclusion, we found that introducing trastuzumab improved the prognosis of HER2-positive AGC patients beyond that of HER2-negative patients. Because other targeted therapies are currently under study, more improvement in the prognosis of HER2-positive AGC patients is expected. Importantly, because HER2-positive patients are a minority of AGC patients (with a prevalence of less than 20 %), further investigation of other key AGC markers is warranted.

Conflict of interest None of the authors have financial or personal conflicts of interest to disclose.

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Preservation of peritoneal fibrinolysis owing to decreased transcription of plasminogen activator inhibitor-1 in peritoneal mesothelial cells suppresses postoperative adhesion formation in laparoscopic surgery

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Background. *Postoperative adhesion formation is regulated by peritoneal fibrinolysis, which is determined by tissue-type plasminogen activator (tPA) and plasminogen activator inhibitor-1 (PAI-1). This study compared peritoneal fibrinolysis and adhesion formation after laparoscopic surgery (LAP) and open surgery (OP).*

Methods. *We divided 154 male rats into 3 groups after cecal cauterization: Control, no treatment; LAP, CO_2 pneumoperitoneum at 5 mmHg for 60 minutes; and OP, laparotomy for 60 minutes. Adhesions were quantified at day 7. The activity and mRNA level of tPA and PAI-1 were determined by enzyme-linked immunosorbent assay in plasma and peritoneal lavage and by real-time polymerase chain reaction in peritoneal mesothelial cells from omentum. We also examined peritoneal fibrinolysis in human gastric cancer patients treated with LAP* (n = *14) or OP* (n = *10).*

Results. *In the animal study, adhesion scores, PAI-1 activity in peritoneal lavage fluid, and PAI-1 mRNA levels in peritoneal mesothelium were significantly greater in the OP group than the control and LAP groups. In the human study, postoperative PAI-1 mRNA levels were significantly greater in the OP group than the LAP group. Additionally, PAI-1 mRNA levels and subsequent adhesion formation were induced by prolonged operative time in the OP group, but not the LAP group.*

Conclusion. *Preservation of peritoneal fibrinolysis owing to decreased PAI-1 expression at the transcriptional level in peritoneal mesothelial cells is associated with suppression of postoperative adhesion formation in LAP. PAI-1 mRNA levels and subsequent adhesion formation were not induced by prolonged operative time in LAP. These results highlight the less invasiveness nature of LAP. (Surgery 2013;153:344-56.)*

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In recent decades, laparoscopic techniques have been integrated into common surgical practices. The benefits of laparoscopic surgery (LAP) in comparison with open surgery (OP) include decreased pain, faster recovery, lesser hospital stay, possibly decreased immunosuppression, and decreased morbidity, such as small bowel obstruction.[1] Previous reports have investigated the influence of CO_2 pneumoperitoneum on the human body[2]; however, the effects of the lower invasiveness of LAP have not yet been fully determined.

Abdominal adhesion formation occurs in 67–93% of abdominal operations, representing a substantial clinical and financial problem.[3] Postoperative adhesions have increased risk of long-term sequelae, including small bowel obstruction, chronic pain, infertility, and difficult reoperative surgery.[4] Because laparoscopic procedures result

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in a more gentle manipulation of tissues, less bleeding, and less contamination with foreign bodies than conventional OP, as well as a smaller skin incision, LAP has potential theoretic advantages over OP in decreasing adhesion formation,[5-8] but very few comparative studies are available to demonstrate this result.[8]

Substantial evidence indicates that formation of adhesions after surgery is determined by the balance between deposition and degradation of fibrin in the early phase of peritoneal tissue repair.[4,9-12] Adhesions are formed when the peritoneum is damaged and the basal membrane of the mesothelial layer is exposed to the surrounding tissues. This injury to peritoneum elicits a local inflammatory response, which leads to the formation of fibrin-rich exudates as part of the hemostatic and traumatic process. In the presence of sufficient peritoneal fibrinolytic activity, resolution of the fibrin strands occurs. If peritoneal fibrinolytic activity is suppressed, however, organization of the fibrin matrix and cellular and vascular ingrowth ensues, leading to fibrous bands between the structures. Fibrinolysis is activated mainly by the plasminogen activator (tPA) and is inhibited by plasminogen activator inhibitor-1 (PAI-1) in the peritoneal cavity, both of which are produced primarily by peritoneal mesothelial cells.[4,13]

Conventional abdominal surgery is accompanied by a rapid decrease in peritoneal fibrinolytic activity, which may lead to peritoneal adhesion formation.[14] Several studies have investigated the alterations of peritoneal fibrinolysis during LAP.[15-19] These reports indicated that peritoneal fibrinolysis was also disturbed by LAP, similar to conventional OP. A few clinical studies have aimed to clarify the difference in peritoneal fibrinolysis between OP and LAP; however, their results remain controversial.[20-24]

Given this background, we hypothesized that less impairment of peritoneal fibrinolysis after LAP could explain the fewer adhesions compared with OP. The aim of this study was to clarify the peritoneal fibrinolytic activity and subsequent adhesion formation after LAP compared with OP using an animal model and in human patients.

MATERIALS AND METHODS

Animal study. *Animals*: The study was performed using 8- to 10-week-old male F344 rats (CLEA Japan, Tokyo, Japan) that weighed 180–250 g. The animals were housed at a constant room temperature with 12-hour light and dark cycles and were provided standard rodent chow and water ad libitum. The animal study was approved by the Hiroshima University Animal Care and Use Committee.

Rat model of operative adhesion formation: All operative procedures were performed under sterile conditions. We induced intestinal adhesion by cecal cauterization as described previously.[12] Briefly, rats were anesthetized with subcutaneous injection of 50 mg/kg pentobarbital sodium solution. The abdomen was shaved, and then laparotomy was performed with a 1.5-cm anterior midline incision. The cecum was isolated and cauterized using the coagulation mode of bipolar forceps (MERA; 30 W, 500 kHz) for 1 second at 1 point.

Operative interventions: Rats were divided into the following 3 groups after cecal cauterization: Control, LAP, and OP. In the control group, the incision was closed immediately after cecal cauterization without any other intervention. In the LAP group, the abdominal incision was closed after cecal cauterization. Subsequently, an 18-gauge intravenous catheter was inserted into the right lower quadrant, and the peritoneal space was insufflated with CO_2 to a pressure of 5 mmHg for 60 minutes using an automatic inflator (Olympus, Tokyo, Japan). A pneumoperitoneum pressure of 5 mmHg was selected for the laparoscopic model, because a previous experimental study demonstrated that 5 mmHg pressure is the optimal pressure in a rat model to induce the same physiologic changes seen in humans.[25] Another 18-gauge catheter was inserted into the left lower quadrant and connected to a water valve and the insufflation of the gas was balanced with its exit to maintain a constant pressure. At the end of the operation, catheters were removed after release of the inflated gas. In the OP group, the abdominal incision was extended to 4 cm from the xiphoid to pubis after cecal cauterization, and peritoneal cavities were exposed to the air for 60 minutes. Then, the abdominal incision was closed. We also examined pneumoperitoneum or laparotomy for 30 and 120 minutes for both LAP and OP groups to examine the influence of operative time.

Adhesion scoring: The rats were anesthetized and killed 7 days after cecal cauterization, and the abdomen was opened to assess adhesions. Each rat was evaluated according to the following standard scoring system, which has been used widely in this field: 0, no adhesion; 1, one thin filmy adhesion; 2, more than one thin adhesion; 3, thick adhesion with focal point; 4, thick adhesion with plantar attachment or >1 thick adhesion with focal point; and 5, very thick vascularized adhesion or >1 plantar adhesion. Images of adhesion formation

around the cecum were recorded at necropsy without revealing the incision size, and adhesion score was determined by 2 independent observers blinded to the experimental group.

Sample collection and storage: Blood and peritoneal lavage were harvested at the indicated time after cecal cauterization in this model. Blood was collected by aortic puncture. To harvest sufficient peritoneal lavage for examination, 4 mL of sterile phosphate-buffered saline was injected intraperitoneally 0.5 hours before killing. Samples were anticoagulated with 1:10 citrate buffer and centrifuged at 3,000 $\times g$, and supernatants were stored at −70°C until analysis. tPA and PAI-1 activity were measured by enzyme-linked immunosorbent assay (ELISA). In addition, total RNA was extracted from omental tissue, and tPA and PAI-1 mRNA expression levels were assessed by quantitative real-time polymerase chain reaction (RT-PCR). Samples of omental tissue were placed immediately in RNAlater (Ambion) and stored at −70°C until RNA extraction, as described below.

Human study. *Patients and surgery*: We examined prospectively 24 consecutive patients who underwent LAP (n =14) or conventional OP (n =10) for gastric cancer with curative intent from February to July 2011 at the Hiroshima University Hospital. Only patients who underwent partial gastrectomy (distal or proximal gastrectomy) with standard lymph node resection were included. Exclusion criteria were emergency operation, previous history of abdominal surgery (except for appendectomy), metastatic disease, serosal invasion, and positive peritoneal cytology. In our institution, patients who were expected to have a tumor with invasion to the mucosa or submucosa without lymph node metastasis were selected for LAP. For LAP, 5 trocars were inserted for CO_2 pneumoperitoneum at a pressure of 10 mmHg. Removal of the stomach and reconstruction were performed through a 4-cm transverse incision of the left upper abdomen. For conventional OP, surgery was performed through a midline abdominal incision from the xiphoid to navel. All operative procedures were performed by the same surgical team. All patients were staged according to the American Joint Commission for Cancer Staging (AJCC/TNM, sixth edition) system. The study was approved by the local ethics committee, and written informed consent was obtained from all patients.

Sample collection and storage: Blood samples were collected just before (preoperative) and just after the operation (postoperative), and on the first postoperative day (POD1). Blood samples were anticoagulated with 1:10 citrate buffer and then immediately put on melting ice. Plasma was separated by centrifugation at 3,000 $\times g$ and stored at −70°C until analysis. tPA and PAI-1 activity were measured by ELISA. Omental tissues were harvested at the beginning and the end of the operation to extract RNA. Omental tissues were collected from sites demonstrating no mechanical injury, and resected specimens were not included. Samples of omental tissue were immediately placed in RNAlater and stored at −70°C until RNA extraction. tPA and PAI-1 mRNA levels were determined by quantitative RT-PCR.

Isolation and culture of human peritoneal mesothelial cells: For patients in the OP group (n = 3), 5-cm^2 samples of omental tissue were harvested at the beginning and end of the operation, and then human peritoneal mesothelial cells (HPMCs) were isolated as described previously.[26,27] Briefly, pieces of omentum were washed with sterile phosphate-buffered saline and then incubated with shaking in 0.125% trypsin for 10 minutes at 37°C. The tissues were removed, trypsin solution containing free mesothelial cells was centrifuged at 100 $\times g$ for 10 minutes, and then the supernatant was discarded. RNA was extracted immediately from this cell pellet, and tPA and PAI-1 mRNA expression was determined by quantitative RT-PCR. In addition, the obtained cell pellets were confirmed to contain HPMCs by the following method: The cell pellets were suspended in M199 medium (Sigma-Aldrich, St. Louis, MO) supplemented with 10% fetal calf serum, 100 U/mL penicillin, and 2 mmol/L L-glutamine and cultured on 10-cm^2 tissue culture flasks coated with rat type I collagen (Nitta Zeratin Co. Ltd, Osaka, Japan). We then confirmed that HPMCs were cultured 1 week after tissue harvest. Cells were identified as HPMCs by their typical cobblestone appearance when confluent (see Fig 6, *A*), as well as by positive staining for cytokeratin and vimentin plus negative staining for factor VIII on immunocytochemistry, as described previously.[26,27] In this manner, we could determine mRNA levels in peritoneal mesothelium, with minimal contamination by other cell types. We isolated HPMCs from omentum harvested at the beginning and the end of operation in the same way; therefore, we were able to compare mRNA levels between these time points without consideration of phenotypic change owing to isolation.

ELISA: The tPA and PAI-1 activity in plasma and peritoneal lavage was measured with rat and human tPA and PAI-1 activity ELISA kits (Innovative Research, Novi, MI) according to the manufacturer's specifications.

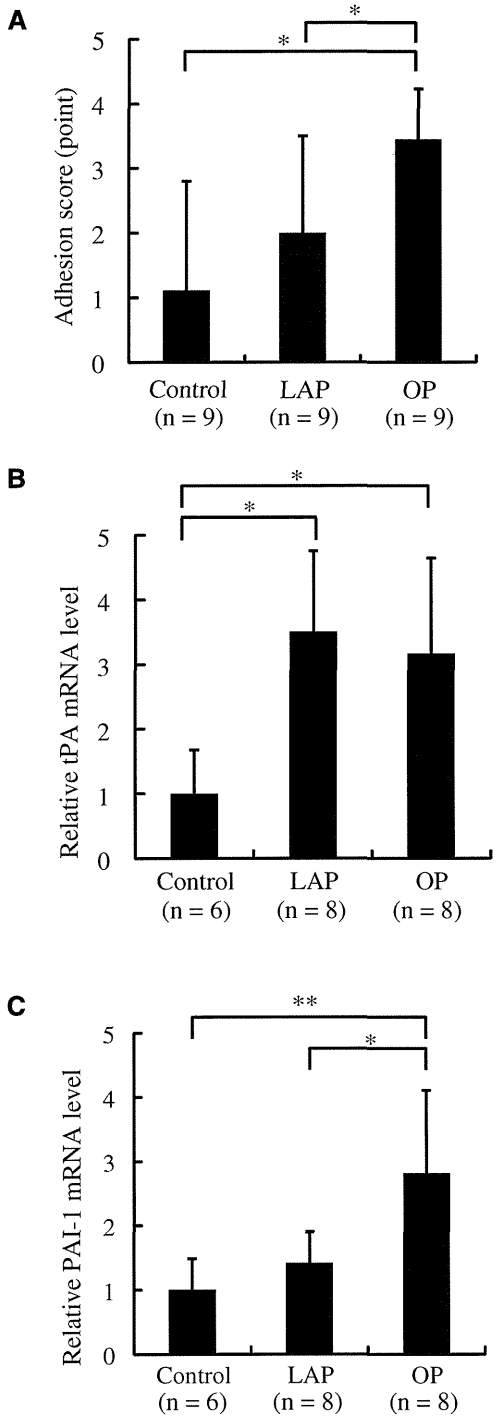

Fig 1. *A*, Adhesion scores of the 3 rat treatment groups at day 7 after cecal cauterization. The adhesion scores of the OP group were greater than those of both the control and LAP groups (*$P < .05$). *B*, Relative tPA mRNA level 6 hours after cecal cauterization for the 3 experimental groups. The tPA mRNA level of the LAP and OP groups was greater than that of the control group (*$P < .05$). No difference was observed between the LAP and OP groups. All data are expressed as a ratio of the mean value for the control group (set at 1). *C*, Relative PAI-1 mRNA level 6 hours after cecal cauterization for the 3 experimental groups. The PAI-1 mRNA level of the OP group was greater than that of both the control (**$P < .01$) and LAP groups (*$P < .05$). All data are expressed as a ratio of the mean value for the control group (set at 1).

Quantitative RT-PCR: Total RNA was extracted from rat and human samples with the RNeasy Mini Kit (Qiagen, Venlo, The Netherlands), and cDNA was synthesized using ReverTraAce qPCR RT kit (Toyobo, Osaka, Japan). We quantified gene expression with TaqMan Gene Expression Assays (Applied Biosystems, Carlsbad, CA). Results were expressed as relative expression standardized to the expression of the gene encoding GAPDH. Specific primers used for quantitative RT-PCR were as follows: PAI-1 (Serpin1; Assay ID Rn01481341_m1), tPA (Plat; Assay ID Rn01482584_m1), and GAPDH (Assay ID Rn99999916_s1) for the animal study; and PAI-1 (Serpin1; Assay ID, Hs01126606_m1), tPA (Plat; Assay ID Hs00263492_m1), and GAPDH (Assay ID Hs99999905_m1) for the clinical study (Applied Biosystems).

Statistical analysis: All data are expressed as the mean value ± standard deviation. Differences between 2 groups were analyzed by the Mann–Whitney test for nonpaired comparison and by paired *t*-test for paired comparison. In all analyses, statistical significance was set at a $P < .05$. All analyses were performed using JMP 8 software (version 8.02, SAS Institute Inc; SAS, Inc, Cary, NC).

RESULTS

Animal study. *Adhesion scoring*: After cecal cauterization, 27 rats were divided into 3 groups: control ($n = 9$), LAP ($n = 9$), and OP ($n = 9$). We killed the rats 7 days later, and then each rat was evaluated according to the previously described adhesion scoring system. No rats died during the experiment or demonstrated intestinal perforation at the time of study. The adhesion scores of the OP group were greater than those of both the control ($P < .05$) and LAP groups ($P < .05$; Fig 1, *A*). These results suggest that the assessment of postoperative adhesion formation in this model is reproducible and optimal to compare fibrinolytic activity between LAP and OP groups.

Measurement of tPA and PAI-1 activity in plasma and peritoneal lavage by ELISA: Another 52 rats were divided into control ($n = 16$), LAP ($n = 18$), and OP ($n = 18$) groups after cecal cauterization. All rats were killed, and blood and peritoneal lavage were collected at the end of the operative

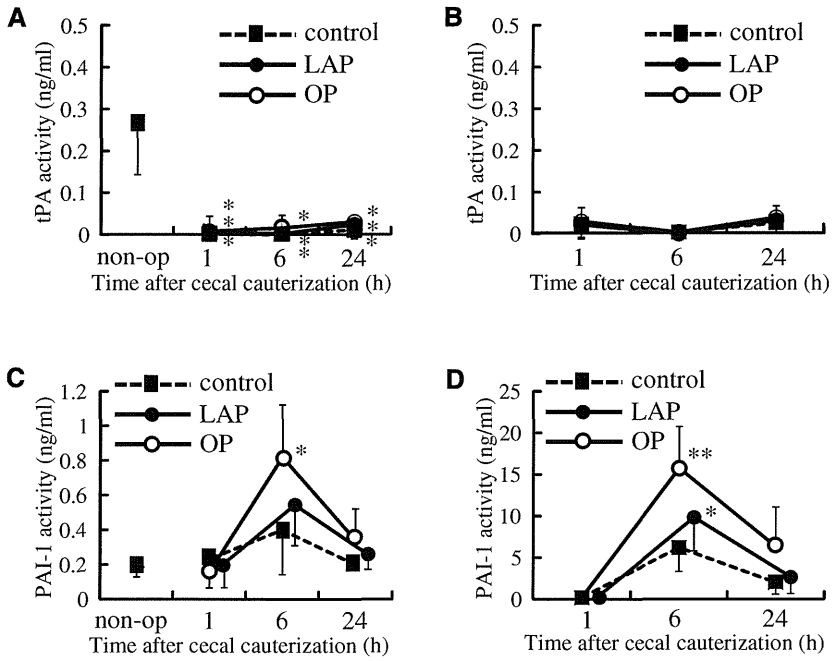

Fig 2. The results of ELISA (animal study). (*A*) Plasma tPA activity. Plasma tPA activity at 1, 6, and 24 hours was decreased compared with that of non-op in the control, LAP, and OP groups (*$P < .05$). No significant differences were observed between groups at each time point. (*B*) tPA activity of peritoneal lavage. The tPA activity in peritoneal lavage was low in all 3 groups at each time point. (*C*) Plasma PAI-1 activity. Plasma PAI-1 activity was increased from non-op to 6 hours in the LAP group ($P < .05$) and OP group ($P < .01$). At 6 hours, PAI-1 activity was greater in the OP group than the control group (*$P < .05$). (*D*) PAI-1 activity of peritoneal lavage. The PAI-1 activity of peritoneal lavage increased from 1 to 6 hours in the control ($P < .05$), LAP ($P < .01$), and OP groups ($P < .01$). At 6 hours, PAI-1 activity was greater in the OP group than in the control (**$P < .01$) and LAP groups (*$P < .05$).

intervention (1 hour after cecal cauterization; $n = 5$ for each group; 6 hours after cecal cauterization, $n = 6$ for the control group, $n = 8$ for the LAP and OP groups; and 24 hours after cecal cauterization, $n = 5$ for each group). Blood and omental tissue were collected as nonoperated control samples from another 5 rats (non-op; $n = 5$).

Plasma tPA activity at 1, 6, and 24 hours was decreased compared with that of non-op controls in all 3 groups ($P < .05$). No differences were observed between each of the 3 groups at each time point (Fig 2, *A*). The tPA activity of peritoneal lavage was low in all 3 groups at all time points (Fig 2, *B*). By contrast, plasma PAI-1 activity increased from non-op to 6 hours in both the LAP ($P < .05$) and OP groups ($P < .01$), and then decreased from 6 to 24 hours. At 6 hours, the PAI-1 activity of the OP group was greater than that of the control group ($P < .05$; Fig 2, *C*). The PAI-1 activity of peritoneal lavage increased from 1 to 6 hours (control group, $P < .05$; LAP and OP groups, $P < .01$), and then decreased at 24 hours. At 6 hours, the PAI-1 activity of the OP group was greater than that of both the control ($P < .01$) and LAP groups ($P < .05$; Fig 2, *D*). The PAI-1 activity of peritoneal lavage demonstrated a dramatic change compared with that of plasma samples, which suggested that induction of PAI-1 activity in the peritoneal cavity was more important for the development of adhesion formation than systemic changes in this model.

Assessment of tPA and PAI-1 gene expression in peritoneal mesothelial cells from omentum: To investigate the transcriptional changes of tPA and PAI-1 in the peritoneal cavity owing to these operative interventions, we examined mRNA expression in omental tissues, which contain high levels of peritoneal mesothelial cells. Peritoneal mesothelial cells are thought to be the primary producer of tPA and PAI-1 in the peritoneal cavity,[4] and the method for extraction of peritoneal mesothelial cells from omental tissue has been established.[26,27] We examined first the time course of PAI-1 mRNA expression changes in response to this operative intervention. We measured PAI-1 mRNA levels from omental tissue extracted from non-op control rats ($n = 5$) and at 1 ($n = 5$), 3 ($n = 5$), 6 ($n = 8$), 9 ($n = 5$), and 24 hours ($n = 3$), and 7 days ($n = 3$) after cecal cauterization in the OP group. The PAI-1 mRNA level gradually increased from

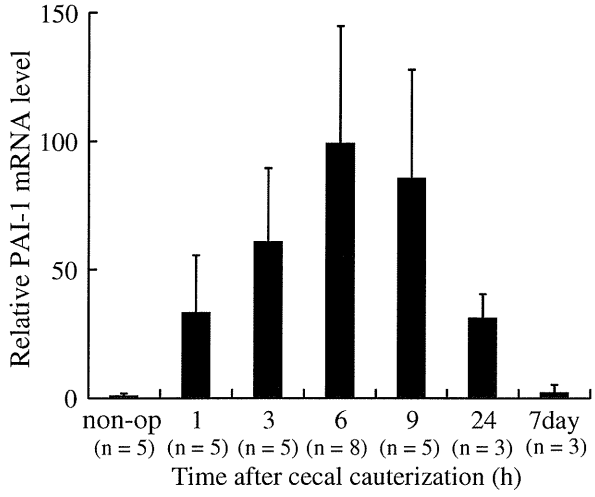

Fig 3. Time course analysis of relative PAI-1 mRNA level after cecal cauterization in the OP group. The PAI-1 mRNA level gradually increased from non-op to 6 hours and then decreased to day 7. All data are expressed as a ratio of the mean value for non-op (set at 1).

non-op to 6 hours, and then gradually decreased up to day 7 (Fig 3).

We then examined omental tPA and PAI-1 mRNA levels at 6 hours in each group ($n = 6$ for the control group; $n = 8$ for the LAP and OP groups). The tPA mRNA level of the LAP and OP groups was greater than that of the control group ($P < .05$; Fig 1, *B*); however, no difference was observed between the LAP and OP groups. By contrast, the PAI-1 mRNA level of the OP group was greater than that of both the control ($P < .01$) and LAP groups ($P < .05$; Fig 1, *C*). This difference was consistent with the adhesion score results, which suggested a correlation between local PAI-1 mRNA expression levels and postoperative adhesion formation. These results indicated that peritoneal fibrinolysis was better preserved in the LAP group owing to decreased transcription of PAI-1 than the OP group, which contributed to the decrease in postoperative adhesion formation in the rat model.

Correlation between peritoneal fibrinolysis and treatment time: We next examined the relationship between peritoneal fibrinolytic activity and treatment time. We performed pneumoperitoneum or laparotomy for 30 and 120 minutes in both LAP (30 minutes, $n = 8$; 120 minutes, $n = 8$) and OP groups (30 minutes, $n = 8$; 120 minutes, $n = 8$) and determined the PAI-1 mRNA levels in omental tissues at 6 hours after cecal cauterization. PAI-1 mRNA levels were greater in the OP group than the LAP group in the 60- and 120-minute (both $P < .05$) treatment groups. PAI-1 mRNA levels were greater in the 120-minute treatment group than the 30-minute treatment group ($P < .01$); however, this induction was not observed in the LAP group (Fig 4, *A*).

Additionally, we examined the relationship between adhesion scores and operative time using another group of rats. We performed pneumoperitoneum or laparotomy for 30 and 120 minutes for both the LAP (30 minutes, $n = 6$; 120 minutes, $n = 8$) and OP groups (30 minutes, $n = 6$; 120 minutes, $n = 8$); adhesion scores were determined at day 7 after cecal cauterization. Adhesion scores were greater in the OP group than the LAP group in the 60- and 120-minute (both $P < .05$) treatment groups. In the OP group, the adhesion score of the 120-minute treatment group was greater than that of the 30-minute treatment group ($P < .05$). This induction, however, was not observed in the LAP group (Fig 4, *B*). PAI-1 gene expression was highly induced in a time-dependent manner in the OP group, resulting in a greater adhesion score at day 7, whereas its expression was suppressed even with the greater operative time in the LAP group, resulting in a lesser adhesion score.

Human study. *Patient characteristics*: We next investigated peritoneal fibrinolytic activity after LAP compared with conventional OP in clinical samples. Patient characteristics are summarized in Table I. No difference was observed in age or gender; however, patients with early stage disease were more common in the LAP group than the OP group ($P < .05$). Increased plasma PAI-1 levels have been shown to associate with increased risk of cardiovascular disease, diabetes mellitus, and the metabolic syndrome. Therefore, we compared preoperative body mass index, serum HbA1c level, total cholesterol, and triglyceride between the 2 groups. No difference was observed in these factors. No patients had an anticoagulant disorder, and prothrombin time did not differ between the 2 groups. Both preoperative and POD1 white blood cell count and C-reactive protein level did not differ between the 2 groups. No patients experienced intraoperative complications. No patients in the LAP group were converted to laparotomy.

tPA and PAI-1 activity in plasma determined by ELISA: Blood samples were collected just before (preoperative) and after the operation (postoperative), and on POD1. The results of ELISA are summarized in Table II. Both preoperative tPA activity and PAI-1 activity did not differ between the 2 groups. Postoperative and POD1 tPA concentration demonstrated no change compared with preoperative levels (Table II). By contrast, PAI-1

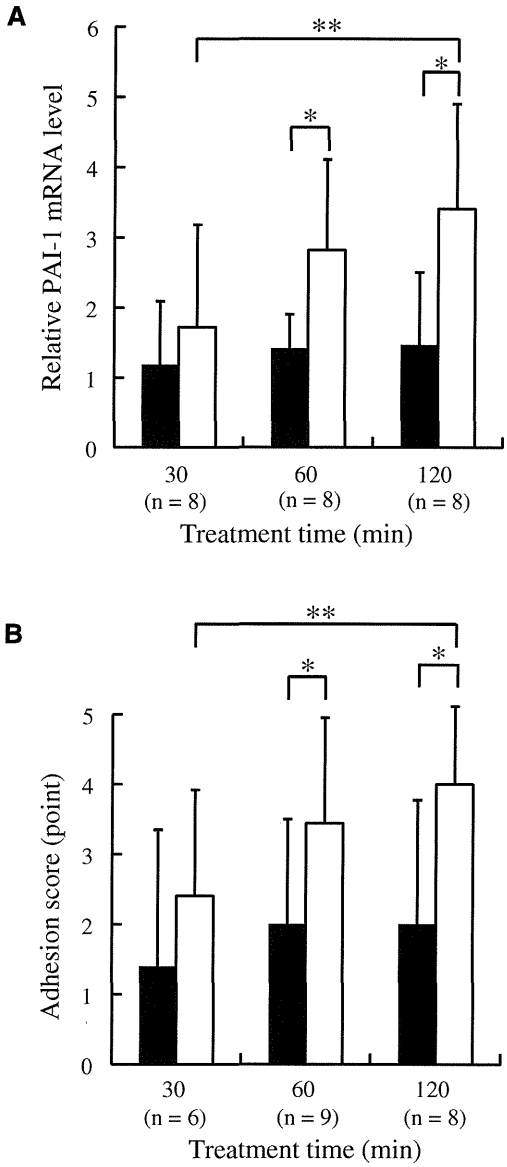

Fig 4. *A*, The correlation between PAI-1 mRNA level and treatment time for LAP and OP groups in the rat cecal cauterization model. PAI-1 mRNA level was greater in the OP group than the LAP group in the 60- (*$P < .05$) and 120-minute (*$P < .05$) treatment groups, respectively. PAI-1 mRNA level was greater in the 120-minute treatment group than the 30-minute treatment group (**$P < .05$) in the OP group; however, this induction was not observed in the LAP group. All data are expressed as a ratio of the mean value for the control group (set at 1). *B*, The correlation between adhesion score and treatment time for the LAP and OP groups. Adhesion scores were greater in the OP group than the LAP group for both the 60- (*$P < .05$) and 120-minute (*$P < .05$) treatment groups. Adhesion scores were greater in the 120-minute treatment group than the 30-minute treatment group in the OP group (**$P < .05$); however, this induction was not observed in the LAP group. *Closed bar* (■), LAP group; *open bar* (□), OP group.

Table I. Patient characteristics

	LAP (n = 14)	*OP (n = 10)*	P *value*
Age	60 ± 15	69 ± 10	NS
Gender (male/female)	7/7	4/6	NS
BMI	23 ± 3	22 ± 3	NS
Stage I/II/III	13/1/0	5/3/2	<.05
TG	126 ± 63	126 ± 85	NS
T-Chol	214 ± 37	192 ± 36	NS
HbA1c	5.6 ± 0.8	5.6 ± 0.4	NS
PT	99 ± 8	102 ± 11	NS
WBC count (preoperative)	6,300 ± 1,900	5,600 ± 1,300	NS
CRP (preoperative)	0.05 ± 0.06	0.08 ± 0.08	NS
WBC count (POD1)	8,900 ± 2,500	9,200 ± 1,600	NS
CRP (POD1)	5.7 ± 2.1	7.0 ± 2.6	NS
Operative time (min)	308 ± 52	224 ± 32	<.001
Bleeding (mL)	48 ± 95	168 ± 138	<.05

All data are expressed as mean values ± standard deviation.
BMI, Body mass index; *CRP*, C-reactive protein; *T-Chol*, total cholesterol; *TG*, triglyceride; *PT*, prothrombin time; *WBC*, white blood cell.

Table II. ELISA results (human study)

	Preoperative	*Postoperative*	*POD1*
tPA activity (IU/mL)			
LAP	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1
OP	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1
PAI-1 activity (ng/mL)			
LAP	8.3 ± 15.5	20.9 ± 32.9*	13.4 ± 14.9*
OP	2.9 ± 4.4	21.8 ± 30.7*	7.3 ± 7.3*

*Versus the preoperative value, $P < .05$.
All data are expressed as mean values ± standard deviation.

activity increased between preoperative and postoperative, and then decreased at POD1 ($P < .05$; Table II); however, the LAP and OP groups did not differ at each time point.

tPA and PAI-1 gene expression in omentum determined by quantitative RT-PCR: Omental tissues were harvested at the beginning and the end of the operation to examine tPA and PAI-1 mRNA expression levels. For both groups, the tPA mRNA level was unchanged after the operation. Both preoperative and postoperative tPA mRNA levels also demonstrated no significant difference between the 2 groups (Fig 5, *A*). By contrast, PAI-1 mRNA levels were significantly increased from pre- to postoperative for both the LAP and OP groups (both $P < .01$). Preoperative PAI-1 mRNA expression did not differ between the 2 groups;

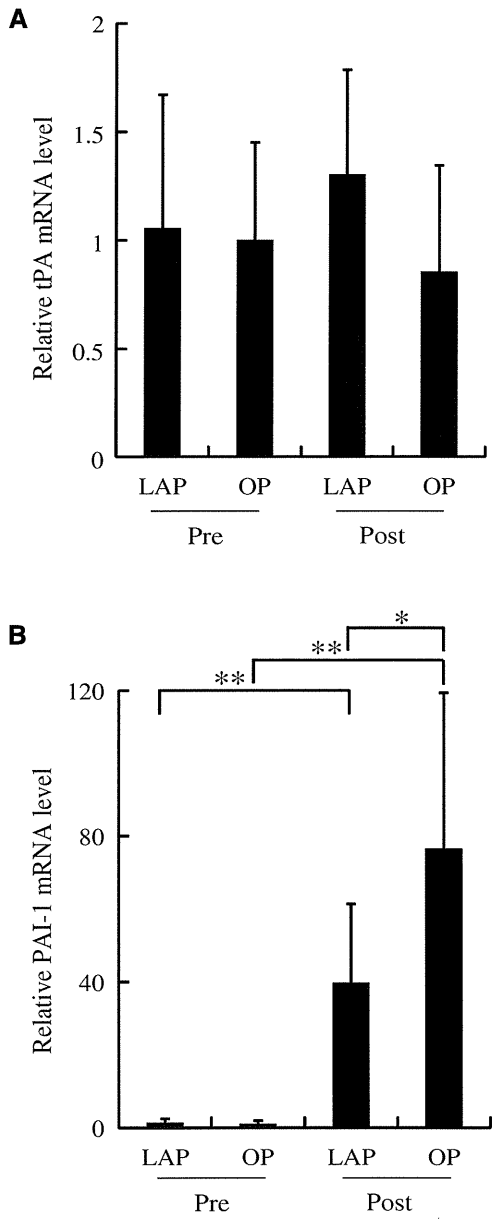

Fig 5. Results of quantitative RT-PCR (human study). *A*, Relative tPA mRNA level. The tPA mRNA expression did not differ between the 2 groups at both pre- and postoperative specimens. All data are expressed as a ratio of the mean preoperative value for the OP group (set at 1). *B*, Relative PAI-1 mRNA level. PAI-1 mRNA level was increased from pre- to postoperative for both the LAP and OP groups (**$P < .001$). The 2 groups did not significantly differ preoperatively; however, the postoperative PAI-1 mRNA level of the OP group was greater than that of the LAP group (*$P < .05$). All data are expressed as a ratio of the mean preoperative value for the OP group (set at 1).

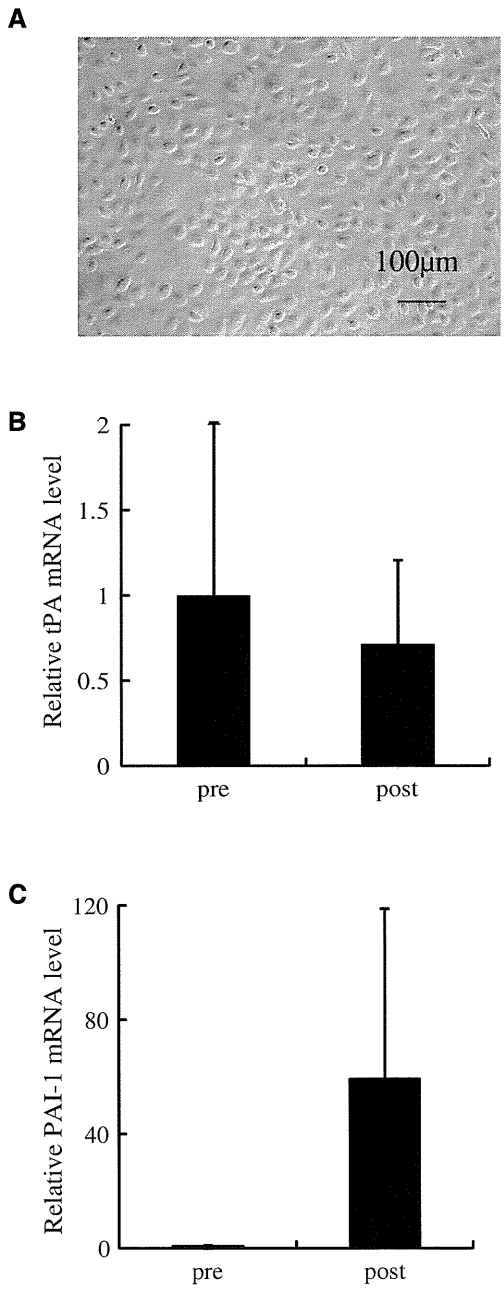

Fig 6. *A*, Phase-contrast micrographs of isolated human peritoneal mesothelial cells (HPMC)s. *B*, Relative tPA mRNA level of isolated HPMCs. The tPA mRNA level did not differ between pre- and postoperative specimens. All data are expressed as a ratio of the mean preoperative value (set at 1). *C*, Relative PAI-1 mRNA level for isolated HPMCs. Postoperative PAI-1 mRNA level tended to be greater than the preoperative level, to a similar extant as that observed in omental tissues. All data are expressed as a ratio of the mean preoperative value (set at 1).

however, the postoperative PAI-1 mRNA level of the OP group was greater than that of the LAP group ($P < .05$; Fig 5, *B*).

tPA and PAI-1 gene expression in isolated HPMCs from omental tissue: To confirm the induction of PAI-1 mRNA in HPMCs from omental tissue in

response to abdominal operation, we harvested 5-cm^2 samples of omental tissue at the beginning and the end of the operation and then extracted HPMCs from each sample from patients in the OP group (n = 3). We only examined the OP group, because obtaining a sufficient amount of omental tissue to extract the HPMCs was difficult in the LAP group owing to the small skin incision. We extracted total RNA from HPMCs and analyzed tPA and PAI-1 mRNA expression by quantitative RT-PCR. tPA mRNA expression did not change after the operation (Fig 6, *B*). Consistent with the induction of PAI-1 mRNA expression in omentum, PAI-1 mRNA expression seemed to be upregulated in HPMCs after the operation (Fig 6, *C*).

Correlation between PAI-1 gene expression and operative time: We investigated the possible correlation of postoperative PAI-1 mRNA levels with several clinical parameters and found that high PAI-1 mRNA levels were correlated with operative time in the OP group (P = .02; r = 0.67; Fig 7). No correlation was observed in the LAP group (P = .56; r = 0.17; Fig 7).

Consistent with our findings in the animal study, these results suggest that achieving a more prolonged hypofibrinolytic state by inhibition of PAI-1 upregulation in peritoneal mesothelial cells during LAP may predispose patients to less intestinal adhesions than OP.

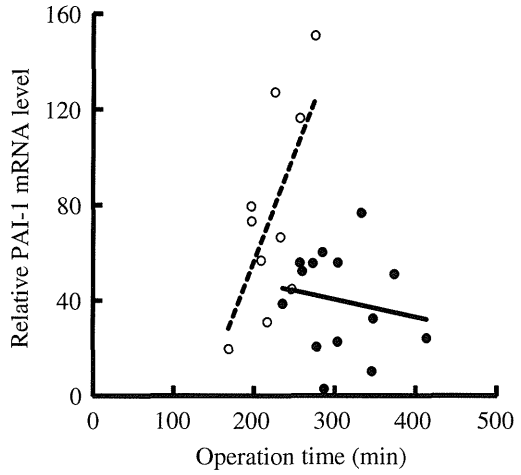

Fig 7. Scatter plots of relative PAI-1 mRNA level versus operative time in the OP and LAP groups. PAI-1 mRNA expression level and operative time were correlated in the OP group (r = 0.67, P = .02). By contrast, PAI-1 mRNA expression level and operative time were not correlated in the LAP group (r = 0.17, P = .56). *Closed circle* (●) and *solid line*, LAP group; *open circle* (○) and *dotted line*, OP group.

DISCUSSION

A considerable body of data now supports the notion that formation of adhesions after abdominal operations is determined by peritoneal fibrinolytic activity.[1,4,12,15,16,18,19,28] The specific condition of a pneumoperitoneum, especially the warm and humid intra-abdominal atmosphere, has been hypothesized to preserve peritoneal fibrinolytic activity in LAP better than in conventional OP[1,8]; however, the extent of this hypofibrinolysis after LAP compared with OP has remained controversial.

The underlying molecular mechanisms that control the peritoneal fibrinolytic system have been clarified in vivo and in vitro. Molinas et al[17] reported that PAI-1 was induced by pneumoperitoneum and identified upregulation of PAI-1 as a mechanism for adhesion formation, because pneumoperitoneum-enhanced adhesions were not observed in *PAI-1* knockout mice. Using an in-vitro pneumoperitoneum model, Bergstrom et al[16] found that cultured HPMCs demonstrated increased synthesis and release of PAI-1 on exposure to CO_2. Ziprin et al[18] reported that incubation of HPMCs with CO_2 enhances mesothelial cell fibrinolytic activity because of a decrease in PAI-1 concentration as compared with standard culture conditions.

Some clinical studies have compared the alteration of peritoneal fibrinolytic activity between LAP and OP and concluded that both operative techniques had similar effects.[21-24] Brokelman et al[24] reported that peritoneal hypofibrinolysis via decreased tPA activity seemed to induce more rapidly during conventional surgery than LAP. All these previous reports analyzed only protein levels from plasma or intraoperative peritoneal biopsies; transcriptional regulation of tPA and PAI-1 in peritoneal mesothelial cells has not been analyzed previously. Because tissue samples to assess the peritoneal fibrinolytic activity in these studies were obtained from peritoneum of the abdominal wall during operation, the quality and quantity of mesothelial cells, which are more abundant in omentum, may have been limited. Moreover, PAI-1 activity and PAI-1 antigen/protein concentration after transcription/translation of the PAI-1 gene may not change significantly, because we detected in the rapid increase of PAI-1 mRNA during operation, presumably owing to the limited duration of the time course in these studies. Further potential biases may have occurred as a result of the operative procedure, skill of the surgeon, blood loss, and the selection of patients suitable for LAP in these clinical studies. By examining transcriptional