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ORIGINAL RESEARCH

High expression of epithelial cellular adhesion molecule in peritoneal metastasis of gastric cancer

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Abstract Intraperitoneally administrated epithelial cellular adhesion molecule (EpCAM) monoclonal antibody is a therapeutic agent in patients with malignant effusion in several types of carcinoma. However, the role of EpCAM in peritoneal metastasis (PM) lesions and primary lesions of gastric cancer (GC) is still unclear. Therefore, in this study, we investigated EpCAM expression in GC patients with PM. We investigated the expression of EpCAM in 35PM lesions and 104 biopsy samples as primary lesions. Immunohistochemical staining was performed using the Ventana Benchmark XT (Roche Diagnostics) system. EpCAM expression was evaluated by calculating the total immunostaining score, which is the product of the proportion score and the intensity score. Overexpression was defined as a total score greater than 4. All PM specimens showed overexpression of EpCAM, and GC cells in both the surface layer and the deep layer of the PM showed a high expression of EpCAM. Meanwhile, in the biopsy sample, the expression of EpCAM ranged from none to strong. The EpCAM score results for PM specimens and biopsy samples were 11.0±2.0 and 6.9±3.9, respectively. The difference between the scores was statistically significant ($P<0.05$). The intraperitoneally administrated EpCAM antibody might have a anti-cancer effect in PM lesions of GC. Additionally, it can be assumed that only GC cells which express a high level of EpCAM might metastasize to the peritoneum.

Keywords Gastric cancer · Peritoneal metastasis · Epithelial cellular adhesion molecule (EpCAM) · Target therapy

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Introduction

Gastric cancer (GC) is the second most common cause of cancer-related death worldwide [1]. Although surgery is the only curative procedure for localized advanced GC, for metastatic or recurrent GC patients, chemotherapy is the only therapeutic approach.

Recently, a number of new drugs to treat GC have become available. Unfortunately, these agents are not particularly effective, resulting in a high recurrence rate, a low survival rate, and a poor prognosis for metastatic or recurrent GC patients [2]. Additionally, GC patients with peritoneal metastasis (PM) have lower survival rates than other GC patients. In a multicenter prospective study, the median survival time was only 3.1 months for GC patients with PM [3]. Thus, another type of treatment for GC patients, particularly those with PM, is required. For example, target therapies that are associated with the expression of a particular gene may open up a new avenue for cancer treatments.

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Table 1 Clinicopathological features of patients

Clinicopathological factors	No. of cases
Gender	
Males	25
Females	10
Average age (range), years	58.6 (22–75)
Borrmann type	
I	0
II	1
III	14
IV	20
Laurens system	
Intestinal type	8
Diffuse type	27
Number of biopsy samples	104

For histopathology typing, gastric cancers were classified as being intestinal or diffuse on the basis of the Laurens system

The epithelial cellular adhesion molecule (EpCAM) is a 39–42-kDa, 314-amino-acid type I transmembrane glycoprotein [4]. EpCAM is detected in the basolateral membrane of the majority of epithelial tissues, and overexpression of EpCAM has been demonstrated in a variety of epithelial cancers [5, 6].

EpCAM has been reported to have effects on cell adhesion, signaling, migration, proliferation, and differentiation, each of which are properties related to metastasis of several types of cancer [7]. In addition, an EpCAM monoclonal antibody, catumaxomab, has been licensed for clinical use in the European Union since 2009 for the intraperitoneal treatment of malignant effusion in patients with EpCAM-positive cells where standard therapy is not available or no longer feasible. Heiss et al. have reported that catumaxomab conferred a puncture-free survival in a prospective randomized phase II/III trial [8]. Furthermore, a subsequent analysis of the report by Heiss et al. revealed that catumaxomab had a significant overall survival benefit to GC patients [9]. However, the expression of EpCAM on the primary lesions and PM lesions of GC is still unclear. Therefore, in this study, we investigated EpCAM expression in GC patients with PM.

Materials and methods

Surgical specimens

Biopsy samples and specimens of PM were obtained from 35 GC patients during upper gastrointestinal endoscopy and staging laparoscopy conducted in our department between 2008 and 2011. All GC patients lacked non-curative factors, such as distant metastasis to liver, lung, or lymph nodes except for PM. In accordance with the Department of Surgery Kinki University Faculty of Medicine policy, written informed consent was obtained from the patients at the time of initial treatment.

Initial treatment

The initial treatment of these patients consisted of single intraperitoneal administration of paclitaxel followed by sequential systemic chemotherapy with S-1 plus paclitaxel. The details of the treatment regimen were described previously [10].

Immunohistochemical study

All sections were placed on the Ventana Benchmark XT (Roche Diagnostics) for detection of the EpCAM oncoprotein. The sections were dewaxed and then subjected to pretreatment with cell conditioning 1 solution (Roche Diagnostics) for 30 min. Sections were then washed with reaction buffer followed by incubation with the mouse monoclonal primary antibody EpCAM (0.1 μg/mL, Vu1D9, Cell Signaling Technology, USA) for 32 min. On-board detection using ultraView Universal DAB kit (Roche Diagnostics), used in accordance with the manufacturer's instructions, was used to detect the location of the primary antibody EpCAM.

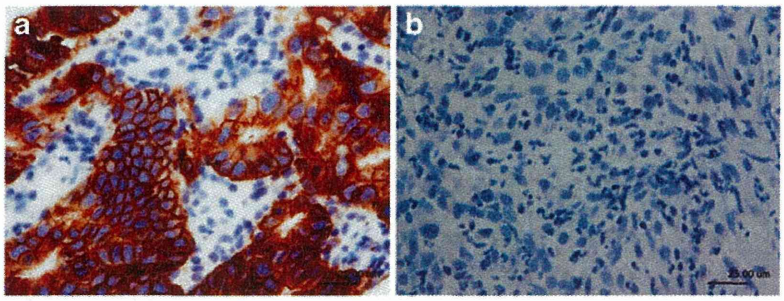

Fig. 1 EpCAM expression in a biopsy sample of gastric cancer. **a** Strong reactivity of EpCAM was visible in most gastric cancer cell membranes in biopsy samples. A representative samples with a score of 12 is shown. **b** Representative sample of gastric cancer cells in a biopsy sample with no reactivity of EpCAM (scored as 0). *EpCAM* epithelial cellular adhesion molecule

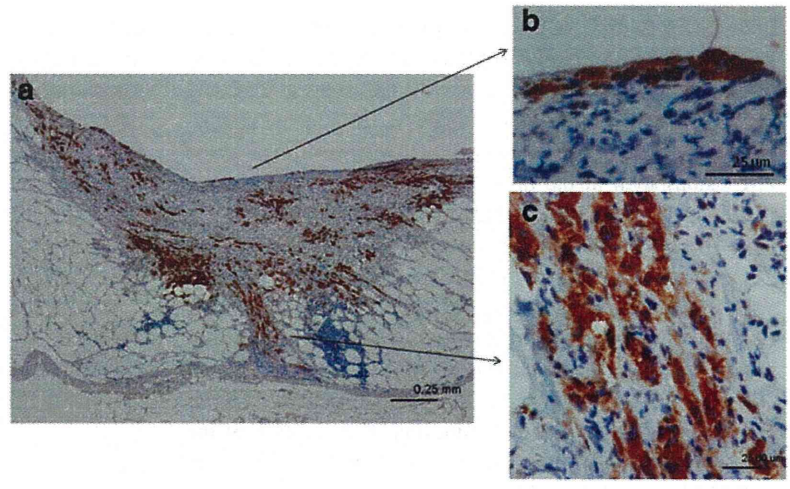

Fig. 2 EpCAM expression of gastric cancer cells in a peritoneal metastasis lesion. **a** High expression of EpCAM is observed in most gastric cancer cells in the peritoneum, scored as 12. **b** Gastric cancer cells show a high expression of EpCAM in the surface layer of the peritoneum. **c** Gastric cancer cells also show a high EpCAM expression in the deep layer of the peritoneum. EpCAM reactivity shows the membrane and cytoplasm of tumor cells. *EpCAM* epithelial cellular adhesion molecule

Immunohistochemical analysis

EpCAM expression was evaluated by calculating the total immunostaining score, which was defined as the product of the proportion score and the intensity score. EpCAM expression was evaluated by the following formula [11]: the proportion score described the estimated fraction of positively stained tumor cells (0, none; 1, <10 %; 2, 10–50 %; 3, 50–80 %; 4, >80 %). The intensity score represented the estimated staining intensity (0, no staining; 1, weak; 2, moderate; 3, strong). The total score ranged from 0 to 12. EpCAM overexpression was defined as a total score greater than 4 [12].

Statistical analyses

The statistical software GraphPad Prism 5 (GraphPad Software Inc, USA) was used to analyze data by Fisher's exact test. A difference of $P<0.05$ was considered as significant.

Results

Patient characteristics

The patients had a median age of 58.6 years (range 22–75 years). There were ten female and 25 male patients. Borrmann III and IV types accounted for the majority. The details of the main clinicopathological features of patients are presented in Table 1. The median survival time of the 35 patients was 23.4 months.

Expression of EpCAM in biopsy samples of gastric cancer

EpCAM expression in 104 biopsy samples from 35 GC patients was determined with immunohistochemical staining. On average, we investigated 2.97 biopsy samples per patient. EpCAM was located on the membrane of GC cells. We observed a diverse range of EpCAM expression intensities. The staining scores of EpCAM ranged from 0 to 12, with an average score of 6.9±3.9. Eighty samples showed overexpression of EpCAM. Figure 1a, b shows representative cases.

Expression of EpCAM in PM of gastric cancer

EpCAM expression in 35PM specimens from 35 GC patients was determined with immunohistochemical staining. EpCAM was located not only on the membrane; diffuse staining was also found in the cytoplasm. Strongly positive-staining tumor cells were found in both the surface layer and the deep layer of the peritoneum. The resulting staining scores of EpCAM ranged from 8 to 12, with an average score of 11.0±2.0. All PM specimens were classified as having EpCAM-overexpressing tumors. Figure 2 shows a representative case.

A significant difference in immunoreactive intensity and average staining score of EpCAM was found between the PM specimens and the biopsy samples ($P<0.05$; Table 2).

Discussion

Between 70 and 100 % of tumor cells in malignant effusions from gastric, ovarian, breast, and colorectal cancer have

Table 2 Overexpression of EpCAM in PM lesions and biopsy samples

	EpCAM overexpression		*P*
	Positive	Negative	
PM lesions	35	0	0.004
Biopsy samples	80	24	

EpCAM epithelial cellular adhesion molecule, *PM* peritoneal metastasis

been found to express EpCAM [13–15]. However, the expression of EpCAM in PM lesions has not been defined. In our study, all specimens of PM with GC showed EpCAM overexpression. This is the first report to reveal these results.

In our study, the expression of EpCAM was stronger in the PM lesions than in the primary lesions. The expression of EpCAM in primary lesions was investigated in biopsy samples. The biopsy samples showed a wide range of EpCAM expression. Conversely, in the PM lesions, almost all GC cells showed a strong EpCAM expression. Furthermore, in vitro studies of EpCAM showed enhanced cell proliferation independent of c-myc and cyclin D_1/E [16, 17].

Additionally, it was reported that EpCAM negatively modulated cadherin-mediated cell adhesion by disruption of the link between α-catenin and F-actin [18]. Furthermore, EpCAM loosened the tight junctions between cells and modulated proliferation, differentiation, and tissue maintenance [19]. Similar phenomena have already been confirmed in breast and renal cancer [19]. In gastric cancer, overexpression of EpCAM might also disrupt cell–cell contact, enabling the cellular migration that is required for metastasis [19]. Thus, only GC cells whose proliferation was enhanced by EpCAM might metastasize to the peritoneum, as this is one of the most frequent metastatic sites of GC.

GC patients with PM have poorer survival outcomes than other GC patients [3]. To improve the survival rate of GC patients with PM, multidisciplinary methods, including intraperitoneal chemotherapy, hyperthermia, and aggressive surgery, have been used to treat PM [20] [21]. However, these trials did not result in a satisfactory clinical outcome. One of the reasons that PM resists multidisciplinary therapy is due to the stem cell characteristics of the cancer cells. Cancer stem cells are responsible for cancer relapse as they are resistant to conventional cancer therapy, such as chemotherapy and radiation [22, 23]. In our results, all PM specimens showed EpCAM overexpression. EpCAM expression is a biologically and clinically relevant characteristic of cancer stem cells from primary GC tissue [24].Therefore, GC cells in PM lesions may have stem cell-like characteristics. The very poor clinical outcomes in GC patients with PM are consistent with these findings.

To improve treatment outcomes of GC with PM, antibody-based cancer therapies are required. Catumaxomab, which is specific for the EpCAM target antigen, is used to treat cancer patients with malignant ascites in the European Union. The clinical benefit of catumaxomab administered by the intraperitoneal route was demonstrated by prospective randomized phase II/ III trials [8]. The antibody can deliver a deadly signal to the cancer cell only by binding to the surface target. However, it seems that the unsatisfactory antitumor effect of catumaxomab on disseminated lesions in the peritoneum is due to the limited penetration of intraperitoneal catumaxomab into the peritoneal surfaces. Additionally, in our study, GC cells in PMs that expressed EpCAM were present not only in the surface layer but also in the deep layer of the peritoneum. Therefore, intraperitoneally administered catumaxomab may only be effective to treat cancer cells in malignant ascites and in the surface layer of the peritoneum.

To further improve treatment outcomes, the investigation of combination therapies comprising systemic chemotherapy plus intraperitoneal catumaxomab and/or intravenously administered catumaxomab may be necessary. Further investigations are required in the future.

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Conflict of interest The authors have no conflicts of interest to declare.

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ORIGINAL RESEARCH

Peritoneal metastatic lesions of gastric cancer exhibit low expression of human epidermal growth factor receptor 2

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Abstract The prognosis of gastric cancer patients with peritoneal metastasis is very poor. Recent findings suggest that use of trastuzumab, a monoclonal antibody-based agent that targets human epidermal growth factor receptor 2 (HER2), may improve the prognosis of gastric cancer patients with HER2 overexpression and/or gene amplification. However, whether these mechanisms of HER2 upregulation are present in gastric cancer patients with peritoneal metastasis is unclear. The status of HER2 expression in a cohort of samples obtained from 35 gastric cancer patients with peritoneal metastasis was investigated using immunohistochemistry and fluorescence in situ hybridization. In 18 cases, we also investigated the influence of induction chemotherapy on HER2 overexpression. The frequency of HER2 overexpression and gene amplification was 2.9 % (1/35) in peritoneal metastatic lesions. There was concurrence in HER2 status in the samples examined prior to and following induction of chemotherapy. Most samples from the gastric cancer patients with peritoneal metastasis did not show HER2 amplification and/or overexpression. Although our study size was small, these results suggest that trastuzumab, which is critically dependent on HER2 expression, might not be an effective agent for these patients. Consequently, other therapeutic approaches for these patients must be developed.

Keywords Gastric cancer · Peritoneal metastasis · Human epidermal growth factor receptor 2 · Trastuzumab

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Introduction

Gastric cancer is the second most common cause of cancer death worldwide. About 1 million people will be diagnosed with gastric cancer per year, and around 700,000 people annually die from their illness [1]. One of the most frequent causes of death from gastric cancer is peritoneal metastasis [2]. Based on the findings of a multicenter prospective study, the median survival time for gastric cancer patients with peritoneal metastasis is around 3.1 months [2].

In a recent randomized, prospective, multicenter clinical Phase III trial, the ToGA trial, efficacy and safety of trastuzumab (a humanized monoclonal anti-human epidermal growth factor receptor 2 (HER2) antibody) for the treatment of HER2-positive gastric cancer patients were evaluated [3]. The findings of this trial showed that trastuzumab conferred an overall survival benefit and was considered a well-tolerated treatment for HER2-positive gastric cancer patients. However, the ToGA trial included patients with peritoneal metastasis, in addition to patients with inoperable locally advanced, recurrent, or metastatic gastric cancer or gastroesophageal cancer. Therefore, the benefits of trastuzumab for gastric cancer patients with peritoneal metastasis remained unclear.

The clinical efficacy of trastuzumab is crucially dependent upon the expression of HER2. Therefore, in this study, we investigated HER2 overexpression and gene amplification in peritoneal metastatic samples obtained from gastric cancer patients.

Materials and methods

Surgical specimens

Peritoneal metastatic samples were obtained from 35 gastric cancer patients at the time of staging laparoscopy between 2008 and 2011. The patients did not have metastases at any other sites (e.g., liver, lung, and lymph nodes). We also investigated 18 primary gastric lesions that were obtained at the time of surgery after the initiation of S-1 (an oral fluoropyrimidine derivative consisting of tegafur, gimestat (which has dihydropyrimidine dehydrogenase-inhibiting activity), and otastat potassium)-based induction chemotherapy. Primary gastric lesions were compared with biopsy samples obtained before induction chemotherapy. In accordance with the policies of the Department of Surgery at Kinki University Faculty of Medicine, written informed consent was obtained from the patients at the time of surgery.

Immunohistochemistry

Freshly resected tissues were fixed overnight at 4 °C in 4 % paraformaldehyde diluted in 0.1 M PBS. The samples were then dehydrated in a series of graded alcohol solutions and embedded in paraffin. Finally, 4-μm thick serial sections were processed for immunohistochemistry, in addition to routine H&E staining.

All sections were placed on the Ventana Benchmark XT (Roche Diagnostics, Tokyo, Japan) system for detection of the HER2 oncoprotein. The sections were dewaxed and then subjected to pretreatment with cell conditioning 1 solution (Roche Diagnostics) for 30 min. The sections were then washed with a reaction buffer followed by digestion with a Protease 1 (Roche Diagnostics) solution for 8 min. The sections were washed again with the reaction buffer and incubated with HER2 mouse monoclonal primary antibody (3.3 μg/ml, Clone SV2-61γ; Nichirei, Tokyo, Japan) for 28 min. On board detection using the ultraView Universal DAB kit (Roche Diagnostics) in accordance with the manufacturer's recommendations was employed to visualize HER2 expression.

Immunohistochemical analysis

Analysis of immunohistochemical findings included evaluation of intensity and staining pattern of HER2 within the tumor cells. As in the ToGA trial [3], scoring for HER2 staining was based on four categories: no staining, or weak staining in fewer than 10 % of the tumor cells (0); weak staining in part of the membrane in more than 10 % of the tumor cells (1+); complete staining of the membrane with weak or moderate intensity in more than 10 % of the cells (2+); and strong staining in more than 10 % of the cells (3+).

Table 1 Clinicopathological information

		Number
Sex	Male	25
	Female	10
Average age (range; years)		58.6 (22–75)
Borrmann type	I	0
	II	1
	III	14
	IV	20
Laurens system type	Intestinal	8
	Gastric	27

Fluorescence in situ hybridization

On the basis of the methodology used in the ToGA trial, the cases that scored 2+ in the immunohistochemical analysis were also examined with fluorescence in situ hybridization (FISH). The HER2 gene was amplified with dual-color FISH probe using a Passvision HER2 DNA probe kit (Vysis, Inc.; Downers Grove, IL, USA) in accordance with the manufacturer's instructions.

Fluorescence in situ hybridization analysis

An image of the region of interest was captured using a CCD camera (ACT-2U; Nikon Corporation, Tokyo, Japan).

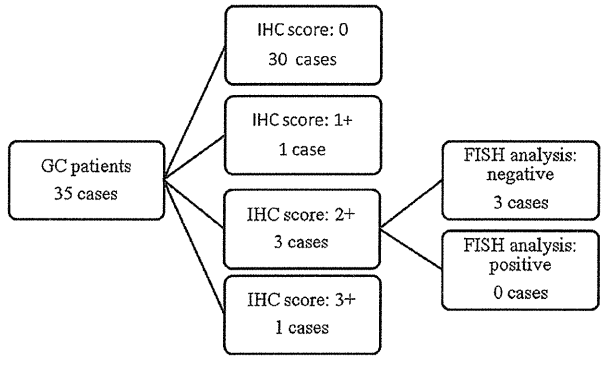

Fig. 1 HER2 overexpression patterns in samples of peritoneal metastatic lesions. HER2 overexpression and gene amplification overexpression were observed in the peritoneal metastatic lesions of only one patient (2.9 %). *HER2* human epidermal growth factor receptor, *GC* gastric cancer, *IHC* immunohistochemistry, *FISH* fluorescence in situ hybridization

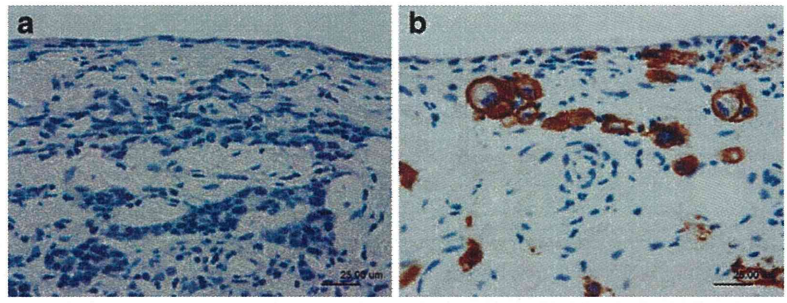

Fig. 2 Representative images demonstrating HER2 expression in peritoneal metastatic specimens from patients with gastric cancer. **a** Tumor rated 0, which shows no HER2 expression in the tumor cells. **b** Tumor rated 3+, showing strong HER2 expression at the tumor cell membrane. No reactivity in activated mesothelial cells was reported. *HER2* human epidermal growth factor receptor 2

A cell was considered to be amplified when a definite cluster or more than ten signals for HER2 was found. Known positive and negative cells were used as controls. Gene amplification was scored when a minimum of 20 cancer cell nuclei exhibited a HER2/CEP17 ratio of greater than 2, or when a HER2 signal cluster was observed.

Results

Patient characteristics

The main clinicopathological features of the patients are presented in Table 1. Borrmann type IV and diffuse forms of gastric cancer accounted for the majority.

Expression of HER2 protein in gastric cancer

HER2 expression status in 71 gastric cancer specimens (35 peritoneal metastatic lesions, 18 primary gastric lesions, and 18 biopsy samples) was determined using immunohistochemistry. The peritoneal metastatic lesions from gastric cancer patients were characterized as follows: 30 cases (85.7 %) were rated 0, one case (2.9 %) was rated 1+, three cases (8.6 %) were rated 2+, and one case (2.9 %) was rated 3+ (Fig. 1). A representative case is shown in Fig. 2.

With respect to the 18 primary gastric lesions that were obtained at surgery after induction chemotherapy, the HER2 expression status in this group was classified according to the following: 15 cases (83.3 %) were rated 0 and the three remaining cases were rated 1+, 2+, and 3+, respectively (Fig. 3). These results were totally consistent with the HER2 expression status of the biopsy samples.

HER2 gene amplification in gastric cancer

As a follow-up, specimens that rated 2+ for HER2 expression were also analyzed by FISH. Three specimens were analyzed. No sample showed HER2 gene amplification. Finally, the frequency of HER2 overexpression and gene amplification in the peritoneal metastatic lesions was 2.9 % (1/35); this lesion was diffuse type.

Discussion

According to our data, the incidence of HER2 of peritoneal metastatic lesions was very low, with 77.1 % (27/35) of the

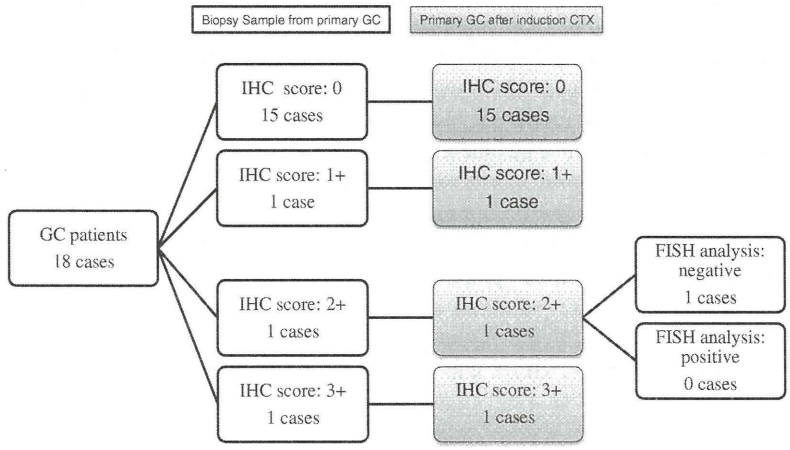

Fig. 3 Influence of HER2 overexpression after induction of chemotherapy. HER2 overexpression and gene amplification overexpression were consistent before and after chemotherapy, as assessed by FISH analysis. *HER2* human epidermal growth factor receptor, *GC* gastric cancer, *IHC* immunohistochemistry, *FISH* fluorescence in situ hybridization

patients exhibiting diffuse-type tumors. Previous reports have indicated that gastric cancers classified as being of the intestinal type are more likely to be HER2-positive than diffuse-type tumors [4] [5]. Our results are consistent with these data. In addition, the prevalence of HER2 expression in diffuse type in the ToGA study was found to be 6 %, and therefore similar to our study [6].

With respect to the ToGA study, 27 % patients who were rated 2+ for HER2 overexpression were gene amplification-positive. In our study, no patients rated 2+ for HER2 overexpression were also found to be positive for HER2 gene amplification. The differences between the ToGA trial findings and the results of our study may be attributed to the histological subtypes reviewed in each study. In the ToGA trial, 75 % of patients had intestinal-type tumors; in contrast, in our study, only 22.9 % of patients had intestinal-type tumors. It has been noted that diffuse-type gastric cancers frequently metastasize to the peritoneum. Thus, on the basis of our findings, it seems likely that gastric cancer with peritoneal metastasis is not associated with HER2 overexpression and/or gene amplification.

The effects of targeted therapy on HER2 expression have been explored in other settings and in other tumor types. For instance, Taucher et al. reported that epirubicin and docetaxel administration as a neoadjuvant therapy for primary breast cancer is not associated with significant changes in HER2 expression [7]. In contrast, in a study of ovarian cancer, Nijman et al. reported an increase in HER2 expression following platinum-based chemotherapy, although their findings were not statistically significant [8]. In the present study, we examined HER2 overexpression in biopsy samples that were obtained before induction chemotherapy and in primary gastric lesions obtained during surgery of 18 gastric cancer patients with peritoneal metastasis that had undergone induction chemotherapy. Our data showed a strong concordance in HER2 expression both before and after chemotherapy.

The findings of the ToGA trial suggested that a monoclonal antibody that targets HER2 may improve the prognosis of advanced and/or recurrent gastric cancer patients with HER2 overexpression and/or gene amplification [3]. Although our study size was small, few gastric cancer patients with peritoneal metastasis showed HER2 overexpression and/or gene amplification. These results suggested that trastuzumab may not be the most effective treatment strategy for gastric cancer patients with peritoneal metastasis. On the basis of these findings, other therapeutic approaches for gastric cancer patients with peritoneal metastasis must be developed and investigated. However, our presented data for the subgroup of gastric cancer with peritoneal metastasis alone indicates that the prevalence of HER2 expression is very low. This does not preclude a higher HER2 expression rate in cases with multiple metastatic sites including the peritoneum. Thus, further investigations are required in the future.

Conclusions

In conclusion, this is the first study to report that almost all gastric cancer patients with peritoneal metastasis examined did not exhibit HER2 amplification and/or overexpression. Therefore, another type of target therapy should be considered for treatment of gastric cancer patients with peritoneal metastasis.

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Conflict of interest The authors declare that they have no conflict of interest.

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ORIGINAL ARTICLE

Phase II Study of Single Intraperitoneal Chemotherapy Followed by Systemic Chemotherapy for Gastric Cancer with Peritoneal Metastasis

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Abstract
Background We conducted a phase II study involving a single administration of intraperitoneal chemotherapy with paclitaxel followed by sequential systemic chemotherapy with S-1+ paclitaxel for advanced gastric cancer patients with peritoneal metastasis.
Methods Gastric cancer patients with peritoneal metastasis were enrolled. Paclitaxel (80 mg/m^2) was administered intraperitoneally at staging laparoscopy. Within 7 days, patients received systemic chemotherapy with S-1 (80 mg/m^2/day on days 1–14) plus paclitaxel (50 mg/m^2 on days 1 and 8), followed by 7-days rest. The responders to this chemotherapy underwent second-look laparoscopy, and gastrectomy with D2 lymph node dissection was performed in patients when the disappearance of peritoneal metastasis had been confirmed. The primary endpoint of the study was overall survival rate.
Results Thirty-five patients were enrolled. All patients were confirmed as having localized peritoneal metastasis by staging laparoscopy. Eventually, gastrectomy was performed in 22 patients. The median survival time of the total patient population and those patients in which gastrectomy was performed was 21.3 and 29.8 months, respectively. The overall response rate was 65.7 % for all patients. The frequent grade 3/4 toxic effects included neutropenia and leukopenia.
Conclusions Sequential intraperitoneal and intravenous paclitaxel plus S-1 was well tolerated in gastric cancer patients with peritoneal metastasis.

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Keywords Gastric cancer · Peritoneal metastasis · Intraperitoneal chemotherapy · Gastrectomy

Introduction

Gastric cancer (GC) is a life-threatening disease worldwide. Recent advances in the treatment of GC have improved clinical outcomes.[1] However, GC patients with peritoneal metastasis (PM) still have a poor overall prognosis.[2] Recently, numerous modalities have been tried in the treatment of PM, such as aggressive surgery, intraperitoneal chemotherapy (IPC), and hyperthermia. However, none of these modalities have shown a satisfactory clinical outcome.[3–5] Consequently, there is no standard treatment for patients with PM.

S-1 (1 M tegafur–0.4 M gimestat–1 M otastat potassium) and paclitaxel (PTX) have a high rate of transition into the peritoneal cavity and a high efficacy against the diffuse type of adenocarcinoma which can easily disseminate.[6,7] Therefore, S-1 and PTX are suitable for PM systemic chemotherapy. In addition, in advanced and/or recurrent gastric cancer patients, several previous trials involving combination chemotherapy with S-1 and intravenous paclitaxel have reported on the safety and efficacy for measurable lesions.[8,9]

PTX has another advantage in the treatment of PM; when administered intraperitoneally it exhibits delayed clearance from the peritoneal cavity because of its high molecular weight and bulky structure. In our recent study we demonstrated the possible effectiveness of PTX for IPC.[10] The advantage of IPC exposure is best expressed as the achievement of a maximal concentration and area under the curve (AUC) ratios of the drug, between the peritoneal cavity and the peripheral blood.[10] Our study showed that the average maximal concentration and AUC ratios for paclitaxel were 1,065:1.[10] However, the clinical effects of intraperitoneal chemotherapy using PTX are unclear.

Therefore, we have developed a new regimen that involves the addition of a single intraperitoneal (IP) administration of PTX to the established systemic chemotherapy regimen of S-1 and PTX for the treatment of PM from GC. In our preliminary study, we confirmed the safety of the regimen.[10] In the present study, we carried out a phase II clinical trial to evaluate the efficacy, response, and safety of this novel multimodal treatment for GC.

Patients and Methods

This study was a prospective phase II study carried out between January 2005 and October 2008. During this period, we performed staging laparoscopy for patients in whom the presence of PM was suspected, for example, a nodular and irregular outer border of the thickened gastric wall, nodules on the peritoneal surface, or a small amount of ascites detected by multi-detector row CT (MDCT). Additionally, with the exception of possible PM, there was a lack of non-curative factors such as distant metastasis to the liver, lung, or lymph nodes. In these patients, the following eligibility criteria that were required for enrolment in this study included: (1) the presence of GC confirmed by histopathology; (2) the presence of PM confirmed by staging laparoscopy; (3) a performance status (Eastern Cooperative Oncology Group [ECOG]) <2; (4) age younger than 75 years; (5) no prior chemotherapy or surgery for gastric or other cancers; (6) adequate bone marrow function (leukocyte count >3,000 ml^{-1} and platelet count >100,000 ml^{-1}), (7) adequate liver function (serum bilirubin level <1.5 $mgdl^{-1}$ and serum transaminase levels less than twice the upper limit of the normal level); (8) adequate renal function, serum creatinine level <1.5 $mgdl^{-1}$; (9) no other severe medical conditions, such as symptomatic infectious disease, intestinal pneumonia, active hemorrhage/bleeding, or obstructive bowel disease; and (10) no current pregnancy or lactation. In accordance with the ethical standards of the committee responsible for human experimentation and with the Helsinki Declaration of 1964, as revised in 1975 and 1983, written informed consent was obtained from the patients before the initiation of treatment and especially before surgery. Patients predicted to be eligible were informed about the therapeutic strategy, emphasizing its potential benefits as well as the possible risk of mortality and morbidity, prior to treatment and especially surgery. Informed consent was given by all patients.

Intraperitoneal Chemotherapy After Staging Laparoscopy

After PM was confirmed at staging laparoscopy, PTX was administered at a dose of 80 mg/m^2.[10] In each patient, PTX dissolved in isotonic saline to a final volume of 1 L was instilled into the peritoneal cavity at the end of the staging laparoscopy. Drainage of the drug solution was not carried out.[10]

Post IPC Systemic Chemotherapy

One week after IPC, S-1 was administered orally twice daily at a dose of 80 mg/m^2/day for 14 consecutive days, followed by 7-days rest. PTX was administered i.v. at a dose of 80 mg/m^2 on days 1 and 8 as previously reported.[8] The treatment course was repeated every 3 weeks until the observation of unacceptable toxicity, disease progression, or responses which might enable a macroscopically curative operation.

Evaluation of Toxicity, Tumor Response, and Indication of Gastrectomy with En Bloc D2 Lymph Node Dissection

Toxicity was measured using the common toxicity criteria of the National Cancer Institute, Version 2.0. In the patients

who had a target lesion, we evaluated the antitumor effects after two and five courses of the treatment and classified them based on the RECIST guidelines. Regarding the patients who had no target lesions, we evaluated the antitumor effects based on the wall thickness of the primary tumor by means of MDCT using the air filling technique. The area in the stomach where the wall thickness was measured corresponded to the area with a biopsy proven tumor mass. A patient was considered a responder in the case of tumor response or a 30 % improvement in wall thickness in one transverse, coronal, and sagittal image and was evaluated using second-look laparoscopy. In cases where there were negative PM findings at second-look laparoscopy, we performed gastrectomy with en bloc D2 lymph node dissection.

Gastrectomy with En Bloc D2 Lymph Node Dissection

The surgical procedure was either total gastrectomy for proximal tumors or subtotal gastrectomy when the primary tumor was located distally in the stomach, with a 5 cm "safe" margin. In all cases, an en bloc D2 lymph node dissection was performed according to the Japanese Gastric Cancer Association guidelines.[11]

Postoperative Chemotherapy

At more than 1 week after the operation, we performed postoperative chemotherapy. Initially one or two courses of weekly PTX,[12] followed by S-1 (80 mg/m^2/day, on days 1–14, every 3 weeks) was administered for more than 1 year or until recurrence was confirmed. Treatment after recurrence was at the physician's discretion.

Statistical Analysis

The JCOG 9205 study reported that the median survival time was 7.1 months (95 % confidence interval (CI), 5.8–8.2 months) in the 5-FU alone arm in patients with advanced and/or recurrent GC.[13] In our study, the median survival time is expected to be shorter than that in the JCOG9205 study owing to the fact that we evaluated patients who had PM. However, the median survival time of the patients whose treatment included an operation is expected to be longer than was the case in the JCOG9205 study. Based on these findings, on the premise that the threshold median survival time is 5 months and the expected median survival time is 9 months, the necessary number of subjects was calculated to be 32 with alpha=0.1 (one-tailed) and beta=0.2. The planned sample size was set at 35, with the consideration of approximately 10 % of patients being ineligible. The accrual time was 3 years and the follow-up time was 2 years after closure of recruitment. The primary endpoint of this study was overall survival. Secondary endpoints were response rate (RR) and safety.

Survival analyses were performed using the Kaplan–Meier method. The survival period was calculated from the first staging laparoscopy date to death or the day of most recent follow-up. Statistical analysis was conducted using the statistical software GraphPad Prism 5 (GraphPad Software Inc, La Jolla, CA, USA).

The clinicopathologic classifications were determined according to the criteria of the TNM Classification of Malignant Tumours, seventh edition. Toxicity and operative complications were measured using the common toxicity criteria of the National Cancer Institute, version 2.0.

Results

During the accrual time, we performed staging laparoscopy in 43 patients. Of these patients, only 35 with PM were enrolled in the current study and fully evaluated. The PM lesions were located mainly on the diaphragm, falciform ligament, and peritoneum. The remaining eight patients could not be enrolled in this study, because they did not have PM. Patient characteristics are listed in Table 1. All patients showed PM at first staging laparoscopy and underwent at least five cycles of systemic chemotherapy. Second-look laparoscopy was performed in 23 patients who were judged as responders according to our criteria. Gastrectomy with lymph node dissection was performed in 22 out of the 23 patients (96.6 %). In the remaining patient who still had PM at the second-look laparoscopy, gastrectomy was not performed. The flow diagram of the treatment protocol is shown in Fig. 1.

Table 1 Patient characteristics and tumor response (n=35)

	Number of patients	
Median age, years (range)	64 (32–75)	
Male/female	23/12	
ECOG performance status 0/1	35/0	
Histological type		
Intestinal	10	
Diffuse	25	
Tumor response		
RECIST guidelines (n=13)		
Complete response	1	8 %
Partial response	7	54 %
Stable disease	3	23 %
Progressive disease	2	15 %
Wall thickness (n=22)		
Over 30 % decrease	15	68 %
Increase	7	32 %

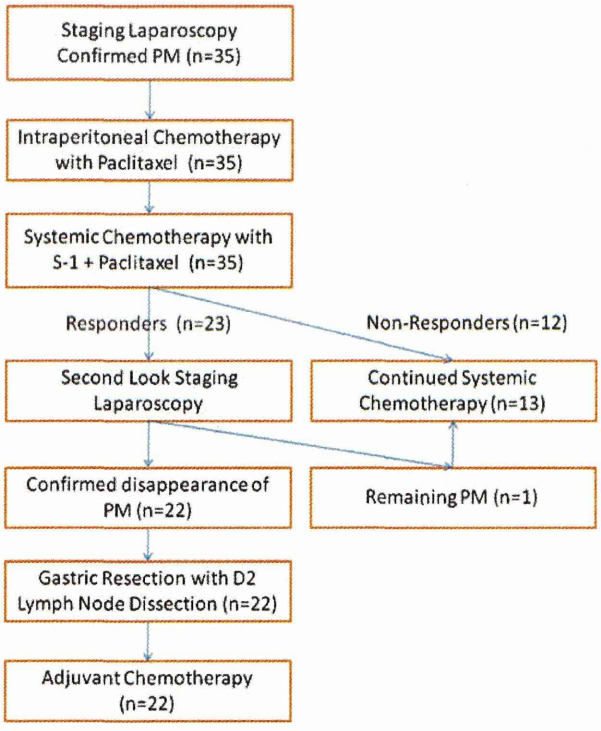

Fig. 1 Flow diagram of the treatment protocol. *PM* patients with peritoneal metastasis

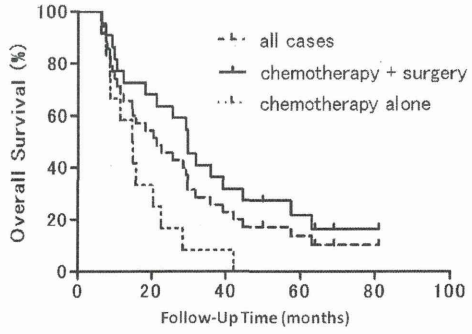

Fig. 2 Kaplan–Meier survival curve for the 35 eligible patients and the patients grouped according to whether or not surgery was carried out. (1) The 35 eligible patients: median survival was 21.3 months with a 1- and 2-year survival rate of 68.6 and 45.7 %, respectively. (2) Chemotherapy+surgery group: median survival was 29.7 months with 1- and 2-year survival rates of 77.3 and 63.6 %, respectively. (3) Chemotherapy alone group: median survival was 14.7 months with a 2-year survival rate of 15.4 %

Survival

At the time of analysis, 31 patients had died and the median follow-up time for the remaining four patients was 69.1 months. The median survival time (MST) of all patients was 21.3 months (95 % CI, 11.4 to 29.8 months), and the 1-year, 2-year, and 5-year overall survival (OS) rates were 68.6 % (95 % CI, 53.2 to 84.0 %), 45.7 % (95 % CI, 29.2 to 62.2 %) and 13.7 % (95 % CI, 2.1 to 25.4 %), respectively. In the patient that underwent gastrectomy, the 1-, 2-, and 5-year OS rates were 77.3 % (95 % CI, 59.8 to 94.8 %), 63.6 % (95 % CI, 43.5 to 83.7 %), and 21.8 % (95 % CI, 4.1 to 39.5 %), respectively, and MST was 29.7 months (95 % CI, 12.3 to 44.6 months). In the patient that received chemotherapy only, the 1- and 2-year OS rates were 53.8 % (95 % CI, 26.7 to 80.9 %) and 15.4 % (95 % CI, 0.0 to 35.0 %), respectively, and the MST was 14.7 months (95 % CI, 7.8 to 20.4 months). There was no patient survival beyond 5 years. The Kaplan–Meier survival curve is shown in Fig. 2.

Response

Thirteen patients had measurable target lesions and the remainder did not. Classification of the patients who had target lesions and were assessed for RR was based on the RECIST guidelines. The RR was 61.5 % (8/13), with one patient showing a complete response, and seven patients showing a partial response. While, out of the 22 patients without a measurable target lesion, a 30 % decrease in wall thickness was seen in 15/22 (68.2 %) (Table 1). Therefore, according of our evaluation of antitumor effects, 23/35 (65.7 %) patients were diagnosed as "responders".

Toxic Reactions

Hematological and non-hematological toxic reactions are listed in Table 2. No patient experienced abdominal pain or any other toxicity related to IPC. During IPC, a grade 3 toxicity reaction was noted in three patients (8.6 %). There were no grade 4 toxicity reactions. However, during systemic chemotherapy the grade 4 toxic reaction of neutropenia was observed in two patients. Frequent grade 3/4 toxic effects included leukopenia (5.7 %), neutropenia (20 %), alanine aminotransferase (ALT) elevation (2.9 %), and bilirubin (2.9 %). There were no treatment-related deaths.

Outcome of Second-Look Laparoscopy

The 23 patients that we diagnosed as responders underwent second-look laparoscopy. Unfortunately, only one patient who was judged as a responder due to a change in wall thickness remained with PM. Therefore, radical resection of all gross and microscopic disease (R0) after induction chemotherapy was accomplished in 22 patients.

Surgical Outcome

Gastrectomy was performed in 22 patients, including total gastrectomy in 19 and distal gastrectomy in three. In almost

Table 2 Adverse events associated with intraperitoneal and systemic chemotherapy

Number of patients (%)

	Intraperitoneal chemotherapy					Systemic chemotherapy				
Grade (CTCAE v2.0)	1	2	3	4	3/4	1	2	3	4	3/4
Hematological toxicities										
Anemia	7 (20)	5 (14.2)	2 (5.7)	0 (0)	2 (5.7)	20 (57.1)	9 (25.7)	0 (0)	0 (0)	0 (0)
Leucopenia	4 (11.4)	3 (8.6)	1 (2.9)	0 (0)	1 (2.9)	7 (20)	10 (29)	2 (5.7)	0 (0)	2 (5.7)
Neutropenia	4 (11.4)	2 (5.7)	1 (2.9)	0 (0)	1 (2.9)	2 (5.7)	7 (20)	5 (14.2)	2 (5.7)	7 (20)
Thrombocytopenia	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
AST elevation	2 (5.7)	1 (2.9)	0 (0)	0 (0)	0 (0)	6 (17.1)	0 (0)	0 (0)	0 (0)	0 (0)
ALT elevation	4 (11.4)	0 (0)	1 (2.9)	0 (0)	1 (2.9)	6 (17.1)	1 (2.9)	1 (2.9)	0 (0)	1 (2.9)
Bilirubin	1 (2.9)	3 (8.6)	0 (0)	0 (0)	0 (0)	6 (17.1)	3 (8.6)	1 (2.9)	0 (0)	1 (2.9)
Creatinine	0 (0)	1 (2.9)	0 (0)	0 (0)	0 (0)	0 (0)	1 (2.9)	0 (0)	0 (0)	0 (0)
Non-hematological toxicities										
Fatigue	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	6 (17)	2 (6)	0 (0)	0 (0)	0 (0)
Anorexia	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	13 (37)	2 (6)	0 (0)	0 (0)	0 (0)
Nausea/vomiting	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	6 (17)	2 (6)	0 (0)	0 (0)	0 (0)
Diarrhea	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	4 (11)	1 (2.9)	0 (0)	0 (0)	0 (0)
Abdominal pain	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Neuropathy	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	7 (20)	0 (0)	0 (0)	0 (0)	0 (0)

all patients, we found a decrease in the size of the main tumor at the time of gastrectomy. The operative complication rate was 9 %, including one case of anastomotic leakage and pancreatic fistula. The details of the 22 patients and the postoperative final tumor stage are listed in Table 3.

Postoperative Chemotherapy

Postoperative chemotherapy was initiated in all 22 patients that underwent gastrectomy, and was completed in all patients. The adverse events of the postoperative chemotherapy were relatively mild, and throughout the treatment period, there were no grade 4 toxic effects.

Discussion

In the current study, our new combination regimen showed a 1-year OS rate of 68.6 % with a MST of 21.3 months. Recent studies targeting unresectable or recurrent GC patients have shown a 1-year OS rate of about 50 %. Our survival results are encouraging in that patents with PM are generally considered to show a particularly poor prognosis.

PM is currently treated with systemic chemotherapy as a palliative, not curative therapy.[14] In brief, there are no GC patients with PM that who survived for over 5 years that had only received chemotherapy.[15] Otherwise, R0 resection is indispensable for curing the gastric cancer. Therefore, we must carefully consider the advantages and disadvantages associated with surgery for GC patients with PM. We might perform gastrectomy on patients who exhibit a response to chemotherapy. In our study, the patients with gastrectomy showed a 1-year OS rate of 77.2 % with a MST of 29.7 months. Additionally, three patients who survived beyond 5 years had undergone gastrectomy. To care for the GC patients, R0 resection was required. Therefore, the GC patients in which PM disappeared after chemotherapy might undergo gastrectomy. Consequently, the survival rates of patients who underwent gastrectomy after chemotherapy were better than those of patients who received chemotherapy alone. This finding indicated that our treatment strategy was appropriate for these patients.

Generally, the effects of chemotherapy are determined by tumor response. However, the evaluation of tumor response in GC patients with PM is difficult because they frequently do not have a target lesion. Therefore, we have developed a new evaluation technique for the chemotherapeutic effect using MDCT with the air filling technique. Using this technique, PM was found to have disappeared in 14 out of 15 (93.3 %) patients who were judged as being responders. Thus, our new evaluation technique was useful in these patients.

With regard to intraperitoneal chemotherapy, toxicity reactions were mild, with only grade 3 toxicity reactions being noted in three patients. Therefore, intraperitoneal chemotherapy with PTX was safe in these patients. During

Table 3 Surgery, pathological results, and postoperative complications in 22 patients

	n	%
Type of resection		
Total gastrectomy	19	86.3
Distal gastrectomy	2	9.0
Pancreaticoduodenectomy	1	4.5
R0 resection rate	22	100
D2 lymph node dissection	22	100
Tumor stage		
CR	1	4.5
M	1	4.5
SM	2	9.0
MP	1	4.5
SS	16	72.7
SE	1	4.5
Nodal stage		
N0	10	45.5
N1	2	9.0
N2	5	22.7
N3a	1	4.5
N3b	4	18.2
Postoperative complications		
Anastomotic leakage	1 (Gr. 2)	4.5
Bleeding	0	0
Intestinal occlusion	0	0
Intra-abdominal abscess	0	0
Pancreatic fistula	1 (Gr. 2)	4.5
Pneumonia	0	0
Surgical site infection	0	0
Death resulting from complication	0	0
Any postoperative complication	2	9.0

Gr. toxicity grade according to the Clavien–Dindo classification

systemic chemotherapy, neutropenia was the main toxic effect; it was more frequent and severe with S-1 plus PTX chemotherapy.[8,9] Non-hematological toxicity effects were relatively mild and were similar to those reported in previous studies.[8,9]

In the present study, the postoperative morbidity rate was 9 %. In previous studies, postoperative morbidity of the patients after chemotherapy for advanced gastric cancer has been reported to occur with a frequency of 31–44.9 %.[16–18] These results indicated that our novel multimodal treatment for GC with PM is feasible and effective.

In conclusion, novel multimodal treatment for GC with PM was well tolerated and active in GC patients with PM. This regimen should be evaluated further in a randomized phase III trial.

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Analysis of the clinical factors associated with anal function after intersphincteric resection for very low rectal cancer

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Abstract

Background: Intersphincteric resection (ISR) has been used to avoid permanent colostomy in very low rectal cancer patients. This study aimed to assess the surgical safety and oncologic and functional outcomes of ISR.

Methods: The records of 30 consecutive very low rectal cancer patients who underwent ISR without neoadjuvant therapy were retrospectively analyzed; survival and locoregional recurrence rates were calculated by the Kaplan-Meier method. Incontinence was assessed by a functionality questionnaire and the Wexner score.

Results: The median distance between the distal margin of the dentate line was 10 mm. A total of 12, 4, and 14 patients underwent partial ISR, subtotal ISR, and total ISR, respectively. The mean distal resection margin was negative in all cases, and circumferential resection margin was positive in two cases. Morbidity was 33.3%: anastomotic stricture in seven patients, colonic J-pouch prolapse in two patients, and an anovaginal fistula in one patient. During the median, 56.2-month follow-up period, local, distant, and combined recurrences occurred in four, three, and two patients, respectively. The 5-year overall and disease-free survival rates were 76.5% and 68.4%, respectively. Local recurrence rates were 5.2% for the patients with Tis-T2 tumors as compared with 45.5% for those with T3 tumors ($P = 0.008$). The mean Wexner scores and stool frequencies, 12 months after stoma closure in 19 patients, were 11.5 and 6.6 per 24 h, respectively. Significant differences were not seen in the Wexner scores between partial ISR and subtotal/total ISR (11.8 ± 2.6 and 9.1 ± 5.6). Stool frequency ($P = 0.02$), urgency ($P = 0.04$), and fragmentation ($P = 0.015$) were worse in patients with anastomotic stricture than in those without; there was no symptom improvement in patients with anastomotic stricture.

Conclusions: The anastomotic strictures in patients undergoing ISR may have negatively affected anal function. For total ISR patients, at least, informed consent stating the possibility of a permanent colostomy is necessary.

Keywords: Intersphincteric resection, Very low rectal cancer, Wexner score

Background

Over the last two decades, surgical treatment for patients with very low rectal cancer has radically evolved, allowing permanent colostomy to be avoided in these patients. Reappraisal of the distal margin has allowed increased potency of sphincter-preserving resections. Moreover, total mesorectal excision (TME) [1], coupled with techniques such as end-anal stapling and coloanal anastomosis using the double-stapling technique (DST) [2], can be used to preserve the sphincter without compromising on the oncological results [3-5].

However, when the tumor is located close to the dentate line, conventional anterior resection using the interperitoneal approach with DST may not allow a secure distal resection margin. To resolve this problem, partial or total internal sphincteric resection (ISR) and coloanal anastomosis per anus can be used for safe surgical resection of the tumor [6-11]. ISR has been proposed to achieve distal clearance in selected patients with very low rectal tumors extending to the upper part of the internal sphincter muscle. Furthermore, it has been

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proposed to restore the anal structure, preserve fecal continence, and reduce the numbers of patients requiring a permanent stoma.

ISR has been widely recognized to achieve a safe distal resection margin, which can be as small as 1 to 2 cm [12,13]. However, with this procedure, which involves dividing the rectum between the internal sphincter and the external sphincter or the levator ani, it remains unclear whether a secure circumferential resection margin (CRM) of the tumor can be obtained. Further, partial or total ISR procedures have been shown to possibly interfere with fecal continence [7,8,14-16].

Anal incontinence is considered to influence various factors in patients receiving ISR, including preoperative radiation therapy [17,18], reconstruction methods [9], extent of sphincter preservation [19], tumor level, and height of the anastomosis [20]. Moreover, fecal incontinence-related quality of life (QOL) scores were poorer in ISR patients than the patients with low anterior resection [16]. Although ISR was proposed as an alternative procedure to avoid abdominoperineal resection (APR), a colostomy is a viable option for patients who suffer from fecal incontinence, which offers a definitive cure along with an improved quality of life [21].

To evaluate the feasibility of ISR in very low rectal cancer patients, it is necessary to clarify the oncologic results and functional outcomes related to this procedure. The aims of this study were to evaluate the surgical safety of the procedure, to assess its oncologic and functional outcomes, and to identify factors predictive of anal dysfunction in the absence of radiotherapy.

Methods

Patients

We reviewed the medical charts of all 30 consecutive patients who had undergone ISR for very low rectal adenocarcinoma between April 2001 and August 2010 at the Department of Surgery, Faculty of Medicine, Kinki University. Written informed consent forms concerning this procedure were obtained for all patients in our hospital. In all cases, tumor stage was evaluated before surgery by digital examination; colonoscopy; chest, abdominal and pelvic computed tomography (CT); and pelvic magnetic resonance imaging (MRI). Anorectal manometry was not routinely performed. Preoperative criteria for the exclusion of patients for ISR were clinical T4 tumors, poorly differentiated adenocarcinoma (revealed by biopsy specimens), infiltrating gross appearance of the tumors, and some degree of preoperative incontinence. Among patients with T1 tumors considered for transanal local excision, ISR was proposed for those patients with a risk of lymph node metastases in the case of tumors with adverse pathologic features. Resectable distant metastases were not a preoperative exclusion criterion for ISR, and therefore, ISR was performed in one patient with synchronous liver metastasis.

The histopathological findings and tumor stage classification were based on the Union for International Cancer Control (UICC) TNM classification (seventh edition) [22]. In Japan, preoperative chemoradiotherapy (CRT) for resectable T3 rectal tumors, irrespective of lymph node involvement, was not routinely performed, and none of the patients included in this study had received preoperative CRT or pre or postoperative radiotherapy.

Surgical technique

The principle of the ISR procedure is based on an anatomic dissection plane between the internal sphincter muscle, which is an extension of the muscular layer of the rectum, and the external sphincter muscle. Surgical intervention was commenced with a high ligation of the inferior mesenteric artery using the abdominal approach. The rectum was dissected to the levator ani with TME. Further, the intersphincteric plane was entered from the nearest anorectal junction if possible. If this dissection was technically difficult to perform until a sufficient distal margin was obtained via the abdominal approach, then the transanal approach of the operation was commenced after perineal exposure using a retractor (Lone Star retractor, Lone Star Medical Products Inc, Houston, TX, USA). The distal margin was 1 cm for Tis-T2 tumors, and 2 cm below the inferior extent of the tumor for T3 tumors. Total ISR involved complete excision of the internal sphincter muscle, that is, the distal line of resection was along the intersphincteric groove. For partial ISR, the distal resection line was along the dentate line, and for subtotal ISR, the distal resection line ran from the dentate line to intersphincteric groove [11,19]. If the tumor was close to the external sphincter or the levator ani muscle, additional partial external sphincter resection (ESR) [11] was performed.

The proximal rectal side of the cut edge was immediately closed and irrigated with 1,500 ml of a 5% povidone-iodine solution to reduce the risk of tumor-cell dissemination [7,23]. Then, the dissection was carried out longitudinally along the plane between the internal and external sphincters to reach the abdominal excision. After the rectum was removed through the abdomen, colonic J-pouch and anal anastomosis procedures with interrupted suture were performed. The anastomosis was protected with a diverting loop ileostomy or transverse colostomy in all the patients.

Follow-up and local recurrences

All 30 patients were followed for a median of 56.2 months (range; 13.3 to 168.4 months), and 20 patients were available for follow-up for more than 2 years. All

patients were followed using a standardized protocol, including a clinical examination with digital palpation, and laboratory tests, including tumors markers (carcinoembryonic antigen (CEA), CA-19-9), every 3 months for the first 3 years, and then every 6 months for 2 years, and then once a year. Abdominal and pelvic computed tomography and chest radiography were performed every 6 months for the first 3 years. A colonoscopy was performed 3 or 6 months after surgery for planning stoma closure, and then once every year for 3 years. Most patients with stage III rectal cancer received postoperative chemotherapy with oral tegafur, uracil, and/or folic acid for 6 to 12 months. Local recurrence was defined as the presence of any anastomotic, pelvic, or lateral node recurrences documented either by clinical or pathologic exanimation, irrespective of the presence of distant metastases.

Anal functional assessments

Functional outcomes were assessed using our functional questionnaire. We prospectively collected questionnaires regarding anal function from our patients every 3 months after closure of the diverting stoma. In this questionnaire, patients were asked about stool frequency (number of bowel movements per 24 h), fecal urgency (ability to defer stool evacuation for >15 minutes), stool fragmentation (>2 evacuations in 1 h), dyschesia (taking more than 15 minutes to defecate), nocturnal defecation, use of intestinal transit regulators, and need to wear a pad. Incontinence was assessed by the Wexner continence score [24], and we considered anal function to be poor if the Wexner score was 15 or more at 12 months [17,18]. Anastomotic stricture or occlusion was determined when the surgeon's forefinger could not pass through the anastomotic site 3 months after surgery.

Statistical analysis

Statistical analyses were performed using JMP10 software (SAS Institute Inc., Cary, NC, USA). Overall and disease-free survival were analyzed using Kaplan-Meier curves and the log rank test. For disease-free survival, patients who failed locally, systemically, or both were censored at the time of the first failure.

Univariate and multivariate regression analyses were used to evaluate the impact of age, gender, type of surgery, type of reconstruction, and anastomotic stricture. The changes in anal function between the different groups of patients over time were compared using Wilcoxon signed-rank test, and comparisons between the anastomotic stricture group and the non-stricture groups were performed using the Mann–Whitney U test. Statistical significance was indicated at the P <0.05 level.

Results

Patients and tumor characteristics are shown in Table 1.

During the study period, ISR covered 144 patients (26.3%) who underwent surgery for lower-third rectal cancer, located below the peritoneal reflex, 49 patients of conventional anterior resection with DST, 35 patients of abdominoperineal resection, and 20 patients of local excision. The study population was made up of 30 patients (16 men and 14 women) with a median age of 58.9 years (range, 31 to 75 years); 1 patient (3.3%) had a pTis of a large villous tumor, 8 patients had a pT1 tumor (26.7%), 10 patients had a pT2 tumor (33.3%) and 11 patients had a pT3 tumor (36.7%). According to the UICC TNM classification system, the tumors were classified as stage 0 in 1 patient, stage I in 16 patients, stage IIA in 5 patients, stage IIIB in 5 patients, and stage IVA in 1 patient.

Table 1 Clinicopathological characteristics of patients who received intersphincteric resection (n = 30)

Characteristic	Value
Age, years[a]	60.5 ± 9.9
Histopathological grade[b]	
G1	12
G2	16
Muc	2
Tumor location	
Anterior wall	14
Posterior wall	12
Left wall	1
Right wall	2
Circ	1
Tumor size, cm[a]	3.8 ± 1.5
<4 cm	18
≥4 cm	12
pT stage	
Tis	1 (3.3%)
T1	8 (26.7%)
T2	10 (33.3%)
T3	11 (36.7%)
TNM stage	
0	1
I	16
IIA	5
IIIA	2
IIIB	5
IVA	1

[a]Values denote mean ± SD.
[b]Differentiation of adenocarcinoma: G1 = well differentiated; G2 = moderately differentiated; Muc = mucinous carcinoma. Circ = circumferential tumor.

Surgical and histopathological findings are shown in Table 2.

In this study, partial ISR, subtotal ISR, and total ISR were performed in 12, 4, and 14 patients, respectively. Furthermore, 4 of 11 patients (36.4%) with T3 tumors intraoperatively decided to undergo additional partial ESR. The mean distance between the distal edge of the tumor and the dentate line was 8.9 ± 8.0 mm (range, -3 to 25 mm) in all the patients. Tumor location was significantly different for each ISR procedure (partial ISR, 16.0 ± 4.6 mm; subtotal ISR, 5.0 ± 4.1 mm; total ISR, 3.5 ± 5.1 mm).

Assessment of the fixed surgical specimens revealed that the median distal edge of the tumor was 7 mm (range, 3 to 22 mm), and it was negative in all cases. The median circumferential margin of the tumor was 3 mm (range, 0.5 to 9 mm). The circumferential resection margin was positive (<1 mm) in two patients with T3 tumor without partial ESR. Reconstruction of the colonic J-pouch was performed in 26 patients, and straight coloanal anastomosis was performed in 4 patients due to narrow pelvis or bulky mesocolic fat tissue.

Mortality and morbidity

There was no mortality. Complications were encountered in ten patients (33.3%). Anastomotic leakage occurred in seven patients, who were treated with perianal drainage. The colonic J-pouch prolapsed in two patients who underwent total ISR. One patient had an anovaginal fistula, requiring repair of fistula using perineal muscular rotation flap, and subsequent stoma closure. Anastomotic stricture or complete occlusion of an anastomosis occurred in seven patients. Of these seven patients, five patients required dilation of the anastomosis using finger bougie, endoscopic balloon dilation, or surgical stricture plasty before stoma closure. Two patients suffered complete occlusion of the anastomosis.

Table 2 Differences in clinicopathological characteristics between intersphincteric resection (ISR) procedures

	Partial ISR, (n = 11)	Subtotal ISR, (n = 4)	Total ISR, (n = 14)
Sex			
Male	6	2	6
Female	6	2	8
Type of reconstruction			
Colonic J-pouch	9	4	13
Straight	3	0	1
Combined with partial ESR	0	2	2
Distance between the distal edge of the tumor andthe dentate line, mm[a]	16.0 ± 4.6	5.0 ± 4.1	3.5 ± 5.1
Distal resection margin, mm[a]	8.7 ± 6.0	9.5 ± 10.5	7.2 ± 5.4
CRM, mm[a]	3.2 ± 2.7	4.8 ± 3.1	3.6 ± 2.1
No. of stoma closures[b]	9 (81.8)	2 (50)	8 (57.1)

[a]Values indicate mean ± SD.
[b]Data in parentheses represent percentage values in each group.
CRM = circumferential resection margin; ESR = external sphincteric resection; ISR = intersphincteric resection.

Oncologic results

Local, distant, and combined recurrence occurred in four, three, and two patients, respectively. Six patients died of cancer recurrence. For all patients who received ISR, the 5-year overall and disease-free survival rates were 76.5% and 68.4%, respectively.

The median disease-free interval for six patients with local recurrence was 13 months (range, 8 to 14 months) (Table 3). All of the four isolated local recurrence episodes developed within the first 2 years. All the patients who experienced local recurrence had pT3 tumors, except one patient who had a pT2 tumor. The local recurrence rates were significantly lower in patients with Tis to T2 tumors (5.2%) than in those with T3 tumors (45.5%; $P = 0.008$; Figure 1).

Aspects of stoma closure

Of the 29 ISR patients, excluding 1 with stage IVA disease, 19 (65.5%) underwent stoma closure by February 2010, including 3 patients who had undergone straight anastomosis. The median interval between ISR and stoma closure was 7 months (range, 3 to 14 months). The median follow-up interval after stoma closure was 35 months (range, 4 to 68 months). Nine, two, and eight patients received stoma closure in the partial ISR, subtotal ISR, and total ISR groups, respectively (Table 2).

Definitive stoma closure could not be performed in 11 patients. Of the 11 patients, 5 had insufficient anal condition (complete anastomotic occlusions in 2, prolapse of colonic J-pouch in 2, obvious loose anastomosis in 1). The patients who developed colonic J-pouch prolapse or obvious loose anastomosis had received total ISR. Four patients were diagnosed with distant metastases or local relapse of the disease before stoma closure. Two patients did not undergo stoma closure for social reasons. Three out of four patients with additional partial ESR did not achieve stoma closure because of a colonic J-pouch prolapse or local recurrence.

Evaluation of anal function

Anal function was evaluated in 19 patients who underwent stoma closure. At 12 months after stoma closure, the mean Wexner score for all patients was 11.5 (range, 1 to 19). In the patients with partial ISR, the Wexner scores were improved from 13.0 ± 3.1 at 3 months to 12.1 ± 3.0 at 6 months ($P = 0.04$). In contrast, in the patients with subtotal or total ISR, no significant differences were found between the Wexner scores at 3 months and 6 months (13.0 ± 3.8 and 11.5 ± 4.9,