Relationship between Expression of Vascular Endothelial Growth Factor in Tumor Tissue from Gastric Cancers and Chemotherapy Effects: Comparison between S-1 alone and the Combination of S-1 plus CDDP

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Background: We have reported that vascular endothelial growth factor (VEGF) expression in gastric cancers might be a selective marker between 5-fluorouracil (5-FU) and a combination of 5-FU plus cisplatin (CDDP). In this study, the relationship between VEGF expression and effects of S-1 with and without CDDP is investigated.

Methods: The subjects were 44 patients treated with S-1 (40 mg/m², twice daily, days 1–28, repeated every 6 weeks) and 24 patients treated with S-1 plus CDDP (S-1 40 mg/m², twice daily, days 1–21, CDDP, 60 or 70 mg/m², day 8, repeated every 5 weeks). VEGF expression in pretreatment endoscopic biopsy samples was assessed immunohistochemically.

Results: Median survival times (MST) of the patients treated with S-1 and S-1 plus CDDP were 344 and 388 days. Among evaluable patients, the response rates of patients with VEGF (+) and (−) tumors to S-1 were 40% (6/15) and 54% (13/24), and to S-1 plus CDDP, 79% (15/19) and 80% (4/5). While the survival of patients with VEGF (−) tumors was slightly longer than those with VEGF (+) tumors in the S-1 group (MST, 425 versus 308 days, \( P = 0.42 \)), patients with VEGF (+) tumors survived remarkably longer than those with VEGF (−) tumors in the S-1 plus CDDP group (MST, 570 versus 333 days, \( P = 0.19 \)).

Conclusion: Similarly to our previous study, it is suggested that the effects of adding CDDP to S-1 might be more remarkable in gastric cancer patients with VEGF (+) tumors than in those with VEGF (−) tumors. These results should be confirmed in a large phase III study.

Key words: vascular endothelial growth factor – S-1 – gastric cancer

INTRODUCTION

The prognosis of patients with unresectable and recurrent gastric cancer is still poor. 5-fluorouracil (5-FU)-based chemotherapy has been widely used for advanced gastric cancer, showing a survival benefit compared with best supportive care (1). In randomized phase III trials, the survival benefit of additional cisplatin (CDDP) to 5-FU has not been clarified (2–4). In the phase III study of the Gastrointestinal Oncology Study Group in the Japan Clinical Oncology Group, although combination therapy of 5-FU plus cisplatin (FP) showed a higher response rate and longer time to progression than continuous infusion of 5-FU (5-FUci), the survival with these two regimens were identical and 5-FUci was less toxic than FP (4).

Recently, many chemotherapy regimens including new agents have been developed that show high response rates for advanced gastric cancer (5–8). S-1 is a new oral fluoropyrimidine, consisting of tegafur (FT), 5-chloro-2, 4-dihydropyrimidine (CDHP) and potassium oxonate. The two phase II studies of S-1 for advanced gastric cancer showed a response rate of 45% in total, with low incidences of severe toxicities (9,10). A combination of S-1 plus CDDP showed a very high response rate of 74% in a phase I/II study.
VEGF in chemotherapy for gastric cancer

In Japan, S-1-based regimens have been widely used in clinical practice for gastric cancer and a randomized phase III study comparing S-1 with S-1 plus CDDP is underway.

It has been generally considered that additional CDDP may bring a benefit to some patients with tumors sensitive to CDDP, whereas it may deteriorate the quality of life in patients with tumors refractory to it. Thus, it is necessary to differentiate patients to be treated with or without additional CDDP.

Progress in basic research has revealed many factors and mechanisms implicated in sensitivity and resistance to chemotherapy. In our first report of the phase II study of FP, patients with positive expression of vascular endothelial growth factor (VEGF) in their primary tumors showed a significantly higher response rate than those negative for VEGF (12). In our second report, patients with VEGF (+) tumors showed a higher response rate than those with VEGF (−) tumors after treatment with a combination of irinotecan plus CDDP (13). Moreover, in our third report, patients with VEGF (+) tumors showed a shorter survival than those with VEGF (−) tumors after treatment with 5-FU. While there was no difference in survival between patients with VEGF (+) and (−) tumors after treatment with FP (14). These results suggest that patients with VEGF (+) tumors might receive a greater benefit from chemotherapy containing CDDP than those with VEGF (−) tumors. However, these results should be recapitulated in other cohorts.

In this study, we investigate the relationship between the expression of VEGF and chemotherapy effects of S-1 alone and S-1 plus CDDP in advanced gastric cancer patients to confirm our previous results that VEGF might be a selective marker for the addition of CDDP.

PATIENTS AND METHODS

PATIENT POPULATION

The subjects of this study consisted of two groups. One was 24 of 25 patients enrolled in the phase I/II study of S-1 combined with CDDP (11). The other group was 44 consecutive patients recruited from 99 patients registered to the post-marketing survey of S-1 (15) from the National Cancer Center Hospital East between April in 1998 and March in 2000. The recruitment criteria for the S-1 group was the same as the eligibility criteria of the phase I/II study of S-1 plus CDDP (11): histologically proven gastric adenocarcinoma; age, 20–74 years; performance status 0–2 on the ECOG scale; no prior chemotherapy; and adequate bone marrow, liver, and renal function. Most importantly, endoscopic biopsy samples taken from primary tumors before chemotherapy were available.

TREATMENT SCHEDULE

The treatment schedule with S-1 was oral administration at a dose that did not exceed 40 mg/m² based on the patient’s body surface area (BSA): BSA < 1.25 m², 40 mg; 1.25 m² ≤ BSA < 1.5 m², 50 mg; and BSA ≥ 1.5 m², 60 mg. This was administered twice daily for 28 consecutive days, followed by 2 weeks rest. In the S-1 plus CDDP group, the same dose of S-1 was administered for 21 consecutive days and CDDP at a dose of 60 (18 patients) or 70 (six patients) mg/m² was given intravenously with adequate hydration and repeated every 5 weeks. Both treatments were repeated until disease progression, unacceptable toxicity, or patient refusal.

EVALUATION OF ANTITUMOR EFFECTS

Tumor responses were evaluated according to the classification of the Japanese Research Society for Gastric Cancer (16), using endoscopy, X-ray imaging or CT scanning. The survival time was calculated from the initial date of the therapy to the date of death from any cause or last confirmation of survival.

IMMUNOHISTOCHEMISTRY

Biopsy samples were immunostained as described in our previous studies (12–14). All immunohistochemical examinations were performed on tissue sections from formalin-fixed and paraffin-embedded biopsy materials from primary tumors. Serial 3-μm thick slices were cut, deparaffinized in xylene, dehydrated with graded ethanol and then immersed in methanol with 0.3% H₂O₂ for 20 min to inhibit endogenous peroxidase activity. The sections were treated with 0.05% pepsin in 0.01 N HCl for 20 min at room temperature. After blocking with 10% normal swine serum in phosphate-buffered saline (PBS; blocking buffer) for 60 min, all sections were incubated overnight at room temperature with the primary antibodies (polyclonal; Santa Cruz Biochemistry, CA, USA) diluted in blocking buffer to 1:500. The sections were washed with PBS and then incubated for 1 h with biotinylated secondary antibody diluted to 1:200. After washing with PBS, the sections were incubated with ABC reagent (Vector Laboratories, CA, USA), and the color reaction was developed in 2% 3,3’-diaminobenzidine and 0.3% H₂O₂ in Tris buffer. The sections were then counterstained with hematoxylin or methyl green.

All immunostained specimens were assessed by one investigator (N.B.) who was blinded to all clinical information. The VEGF staining (Fig. 1) was graded as (+) when the intensity of staining in cancer cells was stronger than that in stromal cells, as (+) when they were equal and as (−) when weaker. Patients were defined as positive when more than 20% of all cancer cells in each section were (+) or (+).

STATISTICAL ANALYSIS

Survival curves were calculated by the Kaplan–Meier method and compared with the log-rank test. Patient characteristics and response rates were compared with a χ² test or Fisher’s exact test.
RESULTS

SUBJECTS

Table 1 shows the clinicopathological features of the subjects. The median age was around 60 years in both treatment groups. Forty-three of the 44 patients in the S-1 group and all 24 patients in the S-1 plus CDDP group had a good performance status of one or less. Histologically, 31 patients (70%) in the S-1 group and 15 (63%) in the S-1 plus CDDP group had diffuse type adenocarcinoma. Twenty-eight patients (64%) in the S-1 group and 20 (83%) in the S-1 plus CDDP group had one or less metastatic sites.

EXPRESSION OF VEGF AND CLINICOPATHOLOGICAL FEATURES

Fifteen of the 44 patients (34%) in the S-1 group had VEGF (+) tumors, while the tumor VEGF positive rate was 21% (5/24) in the S-1 plus CDDP group. Table 2 shows the clinicopathological features of patients with VEGF (−) and (+) tumors in the S-1 group and the S-1 plus CDDP group. In the S-1 group, patients with VEGF (+) tumors were significantly younger than those with VEGF (−) tumors, and other factors related to prognosis such as performance status, tumor extent and number of metastatic sites were slightly better in patients with VEGF (+) tumors than in those with VEGF (−) tumors. In the S-1 plus CDDP group, patient characteristics, except age and histological type, were well balanced between VEGF (+) and (−) tumors.

EXPRESSION OF VEGF AND CHEMOTHERAPY RESPONSE

Among the 39 patients (89%) with evaluable lesions in the S-1 group, the response rate was 49% (19/39); the response rate of all 24 patients in the S-1 plus CDDP was 79% (Table 3). In the S-1 group, the response rate of the 24 patients with VEGF (−) tumors (54%) was slightly higher than that of the 15 patients with VEGF (+) tumors (40%) (P = 0.39). In the S-1 plus CDDP group, the response rates of the patients with VEGF (+) and (−) were very similar.

EXPRESSION OF VEGF AND SURVIVAL

The median survival times (MST) for the S-1 and S-1 plus CDDP groups were 344 and 388 days, respectively (Fig. 2). Figure 3 shows the survival curves of the patients with VEGF (+) and (−) tumors in the S-1 group (A) and the S-1 plus CDDP group (B). In the S-1 group, the MST of the 29 patients with VEGF (−) tumors was 425 days and that of the 15 patients with VEGF (+) tumors was 308 days (P = 0.42). In the S-1 plus CDDP group, the survival of the five patients with VEGF (+) tumors was remarkably long (MST, 570 days), while the MST of the 19 patients with VEGF (−) tumors was 333 days (P = 0.19). In the 48 patients with VEGF (−) tumors, the 29 treated with S-1 survived relatively longer than the 19 patients treated with S-1 plus CDDP (MST, 425 days versus 333 days, P = 0.23). For the 20 patients with VEGF (+) tumors, five patients treated with S-1 plus CDDP showed a longer survival than the 15 patients treated with S-1 (MST, 570 days versus 308 days, P = 0.24).

DISCUSSION

In this study, patients were recruited from two sources; one a registry of a post-marketing survey of S-1 (15), the other a phase I/II study of S-1 plus CDDP (11). The response rate and MST of patients treated with S-1 were 49% (19/39 in evaluable patients) and 344 days, and for those treated with S-1 plus CDDP, 79% (19/24) and 384 days, respectively. Two phase II studies of S-1 showed a response rate of 45% in total, with a MST of 9 months. After the above phase I/II study of S-1 plus CDDP, subsequent
studies of similar combination chemotherapy also reported high response rates and long MSTs (17,18). It is considered that the subjects in this study could reflect the general outcomes of gastric cancer patients treated with S-1 alone and S-1 plus CDDP.

VEGF promotes angiogenesis and the permeability of blood vessels and is associated with microvessel counts and metastasis (19–21). It has been reported that VEGF is a marker of poor prognosis after surgical resection in various kinds of malignancy, including gastric cancer (22–30). It seems that cancers producing VEGF may have a more malignant potential than those not producing VEGF. In our previous report, after treatment with 5-FUci, patients with VEGF (–) tumors showed a slightly higher response rate and significantly longer survival than those with VEGF (+) tumors (14). In this study, after treatment with S-1, the patients with VEGF (–) tumors showed a slightly higher response rate and relatively longer survival than those with VEGF (+) tumors. Comparing the characteristics between patients with VEGF (–) and (+) tumors in the S-1 group, there were more patients with favorable prognostic factors such as good performance status and local advance disease in the VEGF (+) subgroup than in the VEGF (–) subgroup. It is speculated that the difference in survival between

Table 2. Expression of VEGF and clinicopathological features

<table>
<thead>
<tr>
<th></th>
<th>S-1</th>
<th>S-1 + CDDP</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>VEGF (–) (n = 29)</td>
<td>VEGF (+) (n = 15)</td>
</tr>
<tr>
<td></td>
<td>VEGF (–) (n = 19)</td>
<td>VEGF (+) (n = 5)</td>
</tr>
<tr>
<td>Age (median)</td>
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<td>52 (28–66)</td>
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<tr>
<td>Gender (M/F)</td>
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<td>9/6</td>
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<td>PS (0/1/2)</td>
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<tr>
<td>Histological type (intestine/diffuse)</td>
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<td>4/11</td>
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<tr>
<td>Resection of primary tumor (−/+ )</td>
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<td>9/6</td>
</tr>
<tr>
<td>Tumor extent (locally advanced, metastatic)</td>
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<td>11/4</td>
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<tr>
<td>No. of metastatic sites (0/1/2/3 or more)</td>
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<td>0/9/6/0</td>
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<tr>
<td>Total</td>
<td>19/5</td>
<td>49</td>
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</table>

VEGF, vascular endothelial growth factor.

patients with VEGF (–) and (+) tumors might be more prominent if the patient background had been well balanced. Thus, it seems that the relationship between VEGF status and chemotherapy effects such as response and survival might be common between 5-FU alone and S-1 alone.

It has been reported that the response rates in the CDDP-containing regimen of the patients with VEGF (+) tumors were higher than in those with VEGF (–) tumors (12,31). In our first report on the phase II study of FP, the response rate in patients with VEGF (+) tumors was significantly higher than in those with VEGF (–) tumors, while there was no difference in survival between patients with VEGF (+) and (–) tumors (12). Then again, patients with VEGF (–) tumors survived longer than those with VEGF (+) tumors after treatment with 5-FUci (14). In this study, because the number of patients treated with S-1 plus CDDP was small, the difference was not statistically significant. The patients with VEGF (+) tumors survived remarkably longer than those with VEGF (–) tumors after treatment with S-1 plus CDDP, while the survival of the patients with VEGF (–) tumor was slightly longer than in those with VEGF (+) tumors after treatment with 5-FU alone. Considering the results of our previous study (5-FU and FP) and this study (S-1 and S-1 plus CDDP), the relationship between VEGF status and the effects of CDDP additional to 5-FU-based drugs seemed to be similar. Moreover, while the patients with VEGF (–) tumors showed a slightly higher response rate than those with VEGF (+) tumors, those of S-1 plus CDDP were similar between VEGF (–) and (+). The difference in response rate between S-1 alone and S-1 plus CDDP of VEGF (+) subgroup was larger than VEGF (–) subgroup. It is speculated that there might be some unknown mechanisms related to sensitivity to CDDP in gastric cancers producing VEGF and that the addition of CDDP might overcome the malignant potential of VEGF (+) tumor patients.

In conclusion, the relationship between VEGF status and chemotherapeutic effects that had been observed in 5-FU-based chemotherapy with and without additional

Table 3. VEGF expression status and response

<table>
<thead>
<tr>
<th>Treatment</th>
<th>VEGF</th>
<th>CR + PR</th>
<th>NC + PD</th>
<th>RR (%)</th>
<th>P</th>
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<tbody>
<tr>
<td>S-1</td>
<td>(–)</td>
<td>13</td>
<td>11</td>
<td>54</td>
<td>0.39</td>
</tr>
<tr>
<td></td>
<td>(+)</td>
<td>6</td>
<td>9</td>
<td>40</td>
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<tr>
<td>Total</td>
<td>19</td>
<td>20</td>
<td>49</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S-1 + CDDP</td>
<td>(–)</td>
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<td>4</td>
<td>79</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td></td>
<td>(+)</td>
<td>4</td>
<td>1</td>
<td>80</td>
<td></td>
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<tr>
<td>Total</td>
<td>19</td>
<td>5</td>
<td>79</td>
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CR, complete response; PR, partial response; NC, no change; PD, progressive disease; RR, response rate.
CDDP may be reproduced using S-1 and S-1 plus CDDP. It is suggested that the effects of adding CDDP to S-1 might be more remarkable in gastric cancer patients with VEGF (+) tumors than in those with VEGF (−) tumors. These results should be confirmed in a large phase III study.

Acknowledgment

The Taiho pharmaceutical company provided us with part of the clinical data from the phase I/II study of S-1 plus CDDP. We are sincerely grateful to Professor Taguchi for kind advice and arranging this study.

Conflict of interest statement

None declared.

References


20. VEGF in chemotherapy for gastric cancer