



Original article

Chemosensitivity test for 5-fluorouracil and 5-chloro-2, 4-dihydropyridine predicts outcome of gastric cancer patients receiving S-1 postoperatively

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Abstract

Background. Chemosensitivity tests have long been discussed but remain a topic of research. In this study, we investigated the correlation between the results of a chemosensitivity test for 5-fluorouracil and 5-chloro-2, 4-dihydropyridine and the clinical outcomes of gastric cancer patients treated with S-1, an oral fluoropyrimidine, as adjuvant chemotherapy.

Methods. For gastric cancer patients, we performed surgical treatment and a lymph node dissection of D2 or more. Afterwards, a chemosensitivity test for 5-fluorouracil and 5-chloro-2, 4-dihydropyridine was performed, using the collagen gel droplet embedded culture drug-sensitivity test (CD-DST), in surgical specimens. All the patients received postoperative adjuvant chemotherapy with S-1 for 1 year, and the overall survival (OS), relapse-free survival (RFS), and adverse events were investigated.

Results. The chemosensitivity test was performed for 27 patients. The growth inhibition rate (IR) was 50% or more (high-sensitivity group) in 59.3% (16 cases) and it was under 50% (low-sensitivity group) in 40.7% (11 cases). The 3-year OS rate was 100% in the high-sensitivity group and 62.34% in the low-sensitivity group. The 3-year RFS rate was 83.33% in the high-sensitivity group and 24.24% in the low-sensitivity group. Thus, the 3-year OS rate and the 3-year RFS rate were higher in the high-sensitivity group than in the low-sensitivity group. No adverse events of grade 3 or greater severity were observed.

Conclusions. The results of the chemosensitivity test were correlated with the patient outcome. Therefore, such results might be useful for individualizing cancer chemotherapy and for determining future indications for postoperative adjuvant chemotherapy.

Key words Chemosensitivity testing · CD-DST · Gastric cancer · Adjuvant chemotherapy · S-1

Introduction

The selection of appropriate anticancer drugs for individual patients is important, and the recent development of numerous new anticancer drugs for the treatment of gastric cancer has increased expectations for improved therapeutic outcomes. Consequently, chemosensitivity tests have been accepted as a highly advanced medical technology, and their usefulness has been examined in nationwide clinical studies [1].

Individuals with tumors of a similar histopathological class can have markedly different clinical drug sensitivity profiles. In vitro drug sensitivity tests have thus been developed to individualize chemotherapy for cancer patients [2, 3].

Of note, a phase III study of surgical treatment alone vs adjuvant S-1 chemotherapy in patients with curative resections of stage II and stage III gastric cancers (Adjuvant Chemotherapy Trial of TS-1 for Gastric Cancer [ACTS-GC]) reported that postoperative adjuvant chemotherapy with S-1 prolonged the survival of stage II and stage III gastric cancer patients [4]. In the present study, we investigated the predictive clinical value of a chemosensitivity test for 5-fluorouracil (5-FU) and 5-chloro-2, 4-dihydropyridine (CDHP) in patients receiving S-1 postoperatively. The study was performed in May 2004 at an institution affiliated with the Department of Surgery, Nippon Medical School.

Patients, materials, and methods

Patients

This study included 65 patients with stage II, III, or IV gastric cancer with curability A or B who had undergone surgical treatment and a lymph node dissection of D2 or more. The 65 patients were divided into a sensitivity-testing group and a non-test group, using the

randomized clinical trial-envelope protocol. All the patients started adjuvant chemotherapy within 6 post-operative weeks. All the patients received two oral doses of 40 mg of S-1 per square meter of body-surface area per day for 4 weeks, followed by 2 weeks of no chemotherapy. Specifically, during the treatment weeks, patients with a body-surface area of less than 1.25 m² received 80 mg daily; those with a body-surface area of 1.25 m² or more but less than 1.5 m² received 100 mg daily; and those with a body-surface area of 1.5 m² or more received 120 mg daily. Patients underwent hematologic testing and assessments of clinical symptoms every 2 weeks. The presence of a relapse was determined using imaging studies, including ultrasonography, computed tomography (CT), gastrointestinal radiography series, and endoscopy. Patients underwent at least one type of imaging study, usually CT, at 6-month intervals during the first 2 years after surgery and at 1-year intervals thereafter until 5 years after surgery. The treatment was administered for 1 year postoperatively. The primary endpoint was the overall survival (OS) period, and the secondary endpoints were the relapse-free survival (RFS) period and the adverse events. Overall survival was defined as the period between operation and death. Relapse-free survival was defined as the period between operation and the first relapse.

Drug sensitivity test

In the sensitivity-testing group, the chemosensitivity test for 5-FU and CDHP was conducted using the collagen gel droplet embedded culture drug sensitivity test (CD-DST) in surgically resected gastric cancer specimens. A portion of each tumor specimen was excised and thin slices were prepared. Each sample was treated with Dispersion Enzyme Cocktail EZ (Kurabo Industries, Osaka, Japan). The resulting cell suspension was inoculated into collagen-coated flasks (CG-flask; Kurabo Industries) and cultured in preculture medium (PCM-1; containing 10% fetal bovine serum [FBS]) at 37°C in 5% CO₂ overnight. Next, the collagen gel was digested with 0.05% collagenase (type I; Sigma-Aldrich Japan, Tokyo, Japan) and viable cancer cells were obtained. Type I collagen (Cellmatrix Type CD; Kurabo Industries), 10× F-12 medium, and a reconstitution buffer were mixed together in ice water at a ratio of 8:1:1. The prepared cancer cell suspension was added to the collagen solution, with a final density of 1 × 10⁵ cells/ml. Three drops of the collagen-cell mixture (30 μl/droplet) were placed in each well of a six-well multiple plate on ice and allowed to gel at 37°C in a CO₂ incubator; the final concentration was about 3 × 10³ cells per collagen gel droplet. Dulbecco's Modified Eagle and F-12 medium (Gibco) containing 10% FBS was overlaid on each well 1 h later and incubated in a CO₂ incubator at 37°C overnight.

Subsequently, the tumor cells in the collagen gel droplet were exposed to the treatment drugs at concentrations corresponding to the area under the drug concentration time curve (AUC) in clinical cases and incubated for 120 h. The drug concentrations were 4.0 μg/ml of 5-FU and 8.0 μg/ml of CDHP. After the removal of the medium containing the anticancer drugs, each well was rinsed twice with 3 ml of Hanks' balanced salt solution (HBSS) each time, overlaid with 4 ml of PCM-2 medium (serum-free medium; Kurabo Industries), and incubated for a further 7 days. At the end of the incubation, neutral red was added to each well at a final concentration of 50 μg/ml and the colonies in the collagen gel droplets were stained for 2 h. Each collagen droplet was fixed with 10% neutral-buffered formalin, washed in water, air-dried, and quantified using image analysis. The growth rates of control incubations were calculated as the image density on day 7/image density on day 1 [5–8]. Cases with an inhibition rate of 50% or higher were judged as exhibiting high drug sensitivity.

Statistical analysis

All statistical analyses were performed using the JMP 8.0 statistical software program (SAS, Cary, NC, USA). The χ^2 test was used for comparisons among groups. The 3-year survival rates were estimated using the Kaplan-Meier method, and the log-rank test was used to compare the curves. *P* values of less than 0.05 were considered statistically significant.

Informed consent and approval

All the patients were informed of their therapeutic options. None of the treatments were performed without first obtaining informed consent. The study protocol conformed to the ethical guidelines established by the Declaration of Helsinki and was approved by the institutional review board of Nippon Medical School.

Results

Chemosensitivity test

Of the 65 patients, 27 and 38 were included in the sensitivity-testing group and the no-test group, respectively. Based on the results of the sensitivity test, 16 and 11 of the 27 patients in the former group were judged as having high and low drug sensitivities, respectively. The growth inhibition rate was 50% or more (high-sensitivity group) in 59.3% of the patients overall, and the mean overall growth inhibition rate was 56.39%. No differences in age, histological type, stage, or curability were observed among the high-sensitivity group, the

Table 1. Background factors of patients

Factors	High sensitivity <i>n</i> = 16	Low sensitivity <i>n</i> = 11	Non-test <i>n</i> = 38	<i>P</i> value
Age (years)	68.7 ± 11.4	70.4 ± 9.0	66.6 ± 9.9	0.4115
Sex (male:female)	15:1	8:3	24:14	0.0425
Histology (tub:por:sig:muc:pap)	9:6:0:0:1	6:5:0:0:0	15:18:2:3:0	0.3173
Stage (II:IIIA:IIIB:IV)	3:5:6:2	1:3:4:3	18:8:8:4	0.1561
Curability (A:B)	4:12	2:9	15:23	0.3037
Average inhibition rate	68.6 ± 10.7%	38.6 ± 13.7%	—	<0.01

tub, tubular adenocarcinoma; por, poorly differentiated adenocarcinoma; sig, signet-ring cell carcinoma; muc, mucinous adenocarcinoma; pap, papillary adenocarcinoma

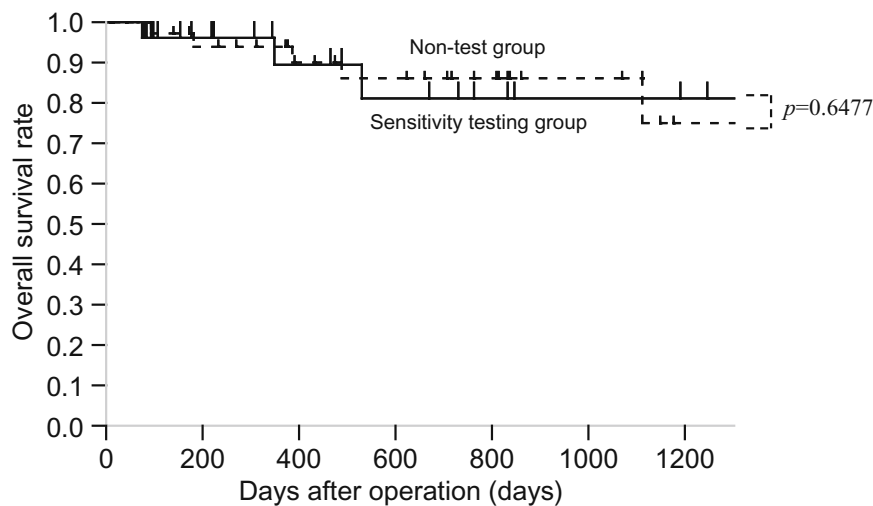


Fig. 1. The 3-year overall survival (OS) rate was 81.17% in the sensitivity-testing group and 85.88% in the non-test group

low-sensitivity group, and the no-test group. In the high-sensitivity group, many of the patients were male (Table 1). The mean growth inhibition rates were $68.6 \pm 10.7\%$ in the high-sensitivity group and $38.6 \pm 13.7\%$ in the low-sensitivity group ($P < 0.01$).

Clinical outcomes of the patients

The median time from operation to follow-up was 844 days. The 3-year OS rate was 81.17% in the sensitivity-testing group and 85.88% in the non-test group ($P = 0.6477$; Fig. 1). The 3-year RFS rate was 45.39% in the sensitivity-testing group and 64.31% in the non-test group ($P = 0.2685$; Fig. 2). No differences in the 3-year OS rate or the 3-year RFS rate were observed between the sensitivity-testing group and the non-test group. Within the sensitivity-testing group, the 3-year OS rate was 100% in the high-sensitivity group and 62.34% in the low-sensitivity group ($P = 0.0464$; Fig. 3). The 3-year RFS rate was 83.33% in the high-sensitivity group and 24.24% in the low-sensitivity group ($P = 0.0117$; Fig. 4). Thus, the 3-year OS rate and the 3-year RFS rate were significantly higher in the high-sensitivity group than in the low-sensitivity group. The mean IR in the patients who relapsed and died was relatively low (29.3%).

Toxicities

All 65 patients were analyzed for adverse events. The grades of adverse events were defined according to the Common Terminology Criteria for Adverse Events, version 3.0 (CTCAE). No adverse events of grade 3 or greater severity were observed (Table 2).

Discussion

In the present study, the results of the chemosensitivity test for 5-FU and CDHP were clearly correlated with the outcome of patients receiving S-1 postoperatively. In this study, patients were divided into a high-sensitivity group and a low-sensitivity group based on the IR of the chemosensitivity test. Significant differences in the OS and RFS were obtained between the high-sensitivity and low-sensitivity groups when IR cutoff values of 20% and 50% were used, respectively (Table 3). However, because the number of patients and the background factors of the patients in each group differed when a cutoff value of 20% was used, we chose 50% as the cutoff value.

Two clinical studies in Japan, the Japanese Clinical Oncology Group (JCOG) 8801 study (mitomycin C

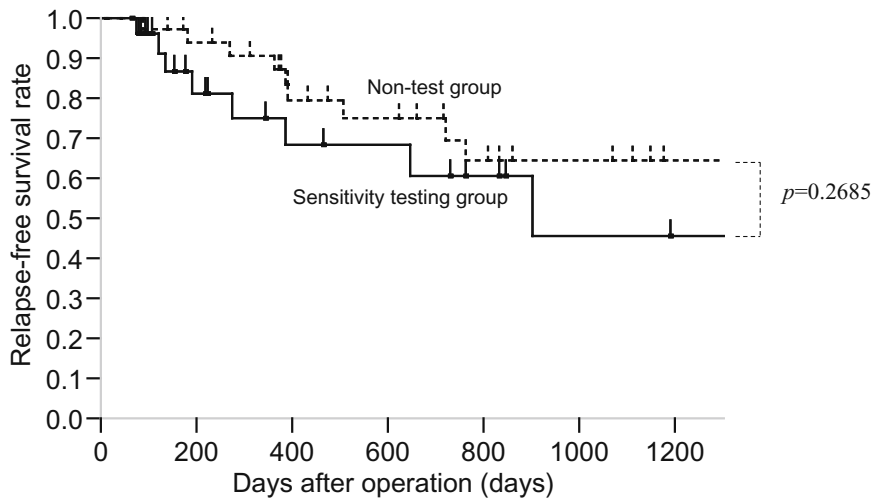


Fig. 2. The 3-year relapse-free survival (RFS) rate was 45.39% in the sensitivity-testing group and 64.31% in the non-test group

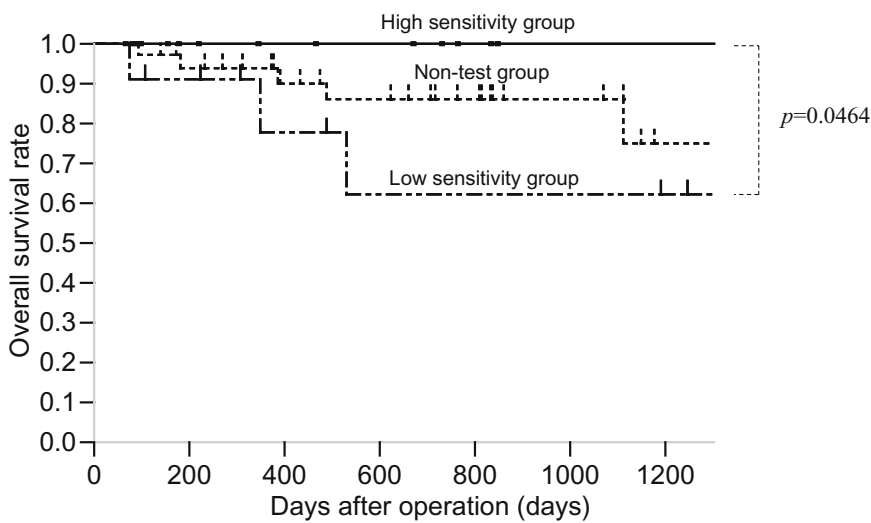


Fig. 3. The 3-year OS rate was 100% in the high-sensitivity group and 62.34% in the low-sensitivity group

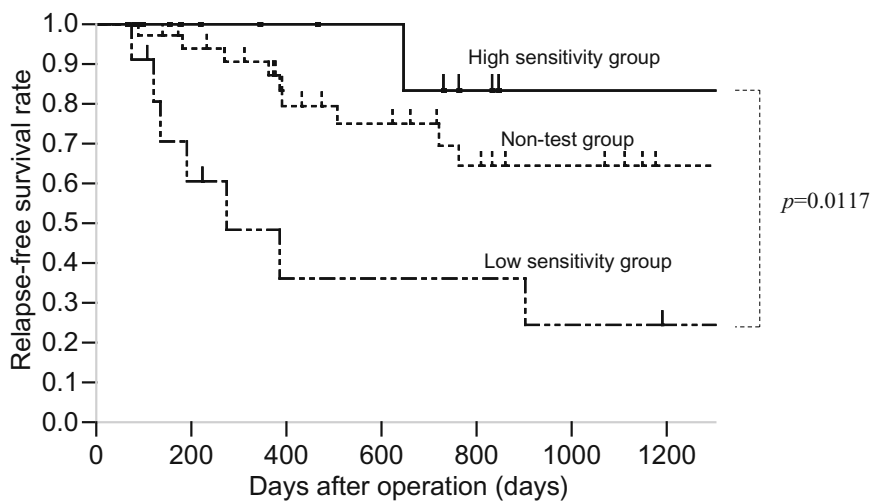


Fig. 4. The 3-year RFS rate was 83.33% in the high-sensitivity group and 24.24% in the low-sensitivity group

Table 2. Adverse events according to treatment

Event	Adjuvant <i>n</i> = 65 (%)	Above grade 3 (%)
Leukopenia	6.2	0
Neutropenia	3.1	0
Anemia	3.1	0
Thrombocytopenia	6.2	0
Elevated AST·ALT level	1.6	0
Elevated total serum bilirubin level	0	0
Stomatitis	3.1	0
Pigmentation	1.6	0
Exanthema	0	0
Anorexia	4.6	0
Nausea·vomitus	1.6	0
Diarrhea	0	0

Grades of adverse events were defined according to the Common Terminology Criteria for Adverse Events (CTCAE) version 3.0

AST, aspartate aminotransferase; ALT, alanine aminotransferase

Table 3. Ratios of high- and low-sensitivity groups, and *P* values of OS and RFS by log-rank test determined at various IR cutoff values

IR cutoff (%)	High sensitivity (<i>n</i>)	Low sensitivity (<i>n</i>)	OS <i>P</i> value	RFS <i>P</i> value
20	25	2	0.0002	0.0100
30	24	3	0.1119	0.3816
40	23	4	0.0097	0.1423
50	16	11	0.0464	0.0117
60	12	15	0.1583	0.1504
70	8	19	0.2190	0.3423
80	2	25	0.3280	0.2252

IR, inhibition rate; OS, overall survival; RFS, relapse-free survival

[MMC] + 5-FU + uracil and tegafur [UFT] vs surgical treatment alone) [9] and the JCOG9206-1 study (mitomycin, fluorouracil, and cytosine [MFC] + 5-FU vs surgical treatment alone) [10], failed to confirm the usefulness of postoperative adjuvant chemotherapy. However, in a post-stratification analysis of the JCOG8801 study, the 5-year survival rate was found to be 78.2% for pT2,3 and pN (+) patients in the adjuvant chemotherapy group, while the rate was 66.6% in the pT2,3 and pN (+) patients treated with surgery alone. Thus, adjuvant chemotherapy appeared to exert some efficacy [11]. In the National Surgical Adjuvant Study Group for Gastric Cancer (NSAS-GC) trial, which was initiated based on data from the post-stratification analysis, surgical treatment alone was compared with the oral administration of UFT in pT2 and pN1,2 patients, and prolonged survival was confirmed in the UFT group [12]. A phase III study comparing the oral administration of S-1 and surgical treatment alone (ACTS-GC) was also conducted in stage II and stage III gastric cancer patients who had undergone curative resections, and a prolongation of survival was confirmed in the S-1 group. At present, oral S-1 is the standard postoperative adjuvant chemotherapy for gastric cancer in Japan [4]. Of note, although

many *in vitro* and *in vivo* methods have been reported to predict the clinical chemosensitivity of tumors in humans [13, 14], their clinical usefulness has not yet been established. However, in joint studies conducted between 1990 and 1993, MMC + UFT was used as the postoperative adjuvant chemotherapy regimen in patients with advanced gastric cancer. Based on the results of chemosensitivity tests conducted in surgically resected specimens, the OS was compared between the group in which the tumor was found to be sensitive to MMC or 5-FU (sensitivity group) and the group in which the tumor was resistant to either drug (non-sensitivity group); the OS rate was found to be significantly higher in the sensitivity group than in the non-sensitivity group [15]. Other reports have also shown significantly more favorable survival periods following adjuvant chemotherapy among patients judged to exhibit sensitivity to the anticancer drugs, compared with patients who did not exhibit sensitivity, according to inferential statistics [16–23]. In addition, similar results have been reported for patients with other types of cancer [24, 25]. Nevertheless, the accuracy of assays for predicting survival needs to be validated in a prospective clinical trial involving patients who have

received the drugs in a postoperative adjuvant setting [26]. Consequently, the validity of sensitivity testing was investigated in the present study by conducting chemosensitivity testing in patients who had received adjuvant chemotherapy with S-1 (i.e., the standard postoperative adjuvant chemotherapy for gastric cancer at present in Japan). The present results revealed that patients with high drug sensitivity had a prolonged survival period and prolonged relapse-free survival (RFS) period, compared with patients who had low drug sensitivity.

In a study reported in 1994 that included stage III and stage IV gastric cancer patients treated with postoperative adjuvant chemotherapy and in whom chemosensitivity testing had been performed, the survival rate in the sensitivity group was significantly more favorable than that in the non-sensitivity group; conversely, no significant difference in the survival rate was observed between the non-sensitivity group and the surgical treatment-alone group [16]. In other words, the postoperative adjuvant chemotherapy might not have had an effect on survival among the patients who were judged as having poor drug sensitivity, and these patients were thus exposed unnecessarily to the risk of adverse reactions associated with chemotherapy. The chemotherapy treatment was also expensive and time-consuming. At present in Japan, S-1 therapy is used as postoperative adjuvant chemotherapy for all patients with stage II and stage III gastric cancer undergoing surgery, based on the results of the ACTS-GC study. However, the chemotherapy might possibly be ineffective and not required in some of these patients. Further investigation using a randomized controlled trial (RCT) is needed. Also, a previous report has suggested that making decisions regarding adjuvant chemotherapy based on the results of chemosensitivity testing could be economically effective in medical settings, allowing medical care costs associated with unnecessary chemotherapy to be avoided [27], thereby suggesting the future usefulness of chemosensitivity tests. On the other hand, several problems remain to be resolved. At present, the chemosensitivity test (CD-DST) for gastric cancer has been approved for health insurance reimbursement in Japan, but the indications for CD-DST are restricted to curability C gastric cancer. However, the CD-DST might also be useful for patients with curability A and B cancers who are scheduled to receive adjuvant chemotherapy. Furthermore, the existing chemosensitivity testing method requires a certain amount of tissue and can be difficult to perform using biopsy materials. A sensitivity-testing method that can be used with biopsy specimens is needed for patients with unresectable gastric cancer. Such tests might improve the therapeutic outcome and allow for individualized treatment by leading to the development of solutions to overcome these problems and tasks.

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