Table 4. Lowest absolute neutrophil count (LNC) during the first treatment cycle

Level	LNC (per mm^3)		Time to LNC (days)		Recovery from LNC (days)	
	Median	Range	Median	Range	Median	Range
1	1860	1287–1884	14	12–22	2	2–6
2	1700	558–1768	14	4–17	3	2–10
3	904	200–1672	14	6–16	8	4–15
4	867	738–996	14	14	8	7–8
5	902	801–1824	14	13–17	6	6–21

was not observed in any patient during any treatment cycle. There was no treatment-related death.

The median numbers of administration cycles were 2 (range 1–8) for level 1, 6 (range 3–11) for level 2, 6 (range 2–13) for level 3, 4 (range 3–6) for level 4, and 3 (range 2–5) cycles for level 5.

An objective tumor response was not observed in the patients at dosage levels 1 and 4. Three patients showed a partial response (PR) at level 2. Two patients showed a complete response (CR) and one patient showed a PR at level 3. Two patients showed a PR at level 5. The overall response rate in all 19 patients with measurable lesions was 42% (95% confidence interval 20–67%). The response rate in pretreated patients was 43% (6/14) and that in chemonaive patients was 40% (2/5). The ascites disappeared in two of three patients without measurable lesions at level 3.

Discussion

Based on the results of phase I and II clinical trials of paclitaxel, the RD was set at 210 mg/m^2 over a 3-week dosing schedule in Japan, and a relatively high tumor response rate of 23% has been reported for advanced gastric cancer [29, 33]. In addition, paclitaxel yielded the same response rate in the second-line setting (23%) as in the first line setting (24%), and non-cross resistance with other anticancer drugs was suggested. In terms of the toxicity, leukopenia and neutropenia of higher than grade 3 were observed in 28% and 58% of patients, respectively. These results are supported by other studies [1, 5]. In recent years, the concept of dose-dense therapy whereby the interval between administrations is shortened to reduce the time for regrowth of neoplastic cells has been proposed [8]. Several clinical studies involving weekly dose-dense therapy of paclitaxel have been performed in lung, breast, and ovarian cancer [16, 24, 27]. Following a phase I clinical trial in 60 patients with advanced cancer who had been treated previously with systemic chemotherapy other than taxanes, the RD was 80 mg/m^2 of paclitaxel weekly, and grade 3 or higher toxicities were rarely observed [16]. In addition, a recent randomized trial comparing two administration methods of paclitaxel with the same dose intensity (conventional 3-week regimen of 200 mg/m^2 and weekly regimen of 67 mg/m^2) in patients with recurrent ovarian cancer revealed equal response rates and overall survival, and reduced toxicities with the weekly schedule [24]. Thus, weekly dosing of paclitaxel has been confirmed to have equal efficacy and lower toxicity than the conventional dosing regimen.

Capecitabine is still under investigation for advanced gastric cancer in Japan. Instead, the immediate precursor to capecitabine, doxifluridine, has been approved for the treatment of advanced gastric cancer. In preclinical evaluations, the therapeutic index of doxifluridine has been shown to be much more than that of 5-FU [4]. In clinical studies, high oral bioavailability of doxifluridine has been noted, and it has shown prominent antitumor activity in patients with breast, colorectal, and gastric cancers [2, 7, 20]. The DLT of doxifluridine is diarrhea. Recently, it has been reported that several anticancer drugs, including paclitaxel, upregulate the expression of dThdPase specifically in tumor tissues and that paclitaxel in combination with doxifluridine shows the synergistic activity in several human cancer xenograft models [26]. Furthermore, the major toxicities of paclitaxel and doxifluridine do not overlap. Therefore, we adopted a weekly dosing regimen of paclitaxel in combination with doxifluridine.

Neutropenia was the most frequently observed toxicity with this combination therapy, and was dose-limiting. However, no neutropenic fever was seen in this study. At dose level 3, one patient experienced a DLT (grade 4 neutropenia for more than 4 days), and five of six patients, including the patient with DLT, exhibited grade 3 or more neutropenia in the first treatment cycle. Based on these results, the investigators and the independent efficacy and safety committee considered it appropriate to stop dose loading at level 3. However, the MTD was not achieved at this level. Then six patients were added to confirm the safety of this dose level. No DLT except for a patient with grade 3 nausea was observed in this additional cohort. Thus, dose escalation was reopened and no grade 4 neutropenia was observed at level 4. Non-hematological toxicity was generally mild. Peripheral sensory neuropathy, one of DLTs of paclitaxel, was well tolerated up to level 4, and diarrhea, a DLT of doxifluridine, was not severe in the initial few cycles at all levels. At level 4, there was only one patient who needed dose reduction due to diarrhea after four treatment cycles. The median numbers of cycles administered were 6 (range 2–13) at level 3 and 4 (range 3–6) at level 4. The main reason for stopping the treatment was disease progression at levels 3 and 4. From

these results, the dosage schedule at level 3 or 4 seems to be highly feasible.

Conventional 3-h infusion of paclitaxel with a 3-week interval combined with infusional 5-FU and cisplatin was studied in Korean group in a phase II trial in advanced gastric cancer [14]. A high response rate of 51% and good tolerability was reported in this study. Recently, it has been reported from Germany that weekly administration of paclitaxel with a combination of 5-FU/folinic acid and cisplatin showed a reduced incidence of hematological toxicity, particularly leukopenia, and other toxicities, apart from a slightly higher incidence of peripheral neuropathy, were also comparable between the weekly regimen and the conventional regimen [11]. The response rate (50%) in this German phase II study in advanced gastric cancer was well maintained with the weekly regimen.

Active oral fluoropyrimidines, such as capecitabine, S-1, and uracil/ftorafur (UFT) plus leucovorin have recently been developed [3, 15, 25]. Based on promising reports, trials are being urgently undertaken in many countries to determine whether 5-FU combined with various agents could be replaced by these new oral fluoropyrimidines. Although most patients in our study had received prior chemotherapy, this doxifluridine and paclitaxel combined therapy yielded a high response rate of 42% (95% confidence interval 20–67%). In addition, elimination of ascites was observed in two of three patients. The efficacy of this combination therapy would also be expected in patients with peritoneal dissemination that is frequently seen in advanced gastric cancer. These results encouraged us to move to further trials.

In conclusion, we performed a phase I clinical trial using a combination of paclitaxel and doxifluridine, and determined the RD as 80 mg/m^2 of paclitaxel on days 1 and 8, and 800 mg/m^2 per day of doxifluridine for 2 weeks in a 3-week treatment schedule. The results of our present study are promising and a phase II clinical trial of this combination therapy is planned in which the safety of the RD will be investigated carefully in the first six or more patients.

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Biological Markers as a Predictor for Response and Prognosis of Unresectable Gastric Cancer Patients Treated with Irinotecan and Cisplatin

Fumio Nagashima[1], Narikazu Boku[1], Atsushi Ohtsu[1], Shigeaki Yoshida[1], Takahiro Hasebe[2], Atsushi Ochiai[2], Yu Sakata[3], Hiroshi Saito[4], Yoshinori Miyata[5], Ichinosuke Hyodo[6] and Masahiko Ando[7]

[1]Division of Digestive Endoscopy and Gastrointestinal Oncology, National Cancer Center Hospital East, [2]Pathology Division, National Cancer Center Research Institute East, Kashiwa, Chiba, [3]Department of Internal Medicine, Misawa City Hospital, Aomori, [4]Department of Medicine, Yamagata Prefectural Central Hospital, Yamagata, [5]Department of Medicine, Saku General Hospital, Nagano, [6]Department of Internal Medicine, National Shikoku Cancer Center, Matsuyama and [7]Health Service, Kyoto University, Kyoto, Japan

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Background: Previously we reported that immunohistochemical examination of p53, bcl-2, glutathione *S*-transferase-π (GST-π), thymidylate synthase (TS) and vascular endothelial growth factor (VEGF) in biopsy samples was a useful method for predicting clinical outcome of gastric cancer patients treated with 5-fluorouracil and cisplatin. Here, we investigated if these biological markers can predict chemoresponse and survival of unresectable gastric cancer patients treated with irinotecan and cisplatin.

Methods: The subjects were 55 unresectable gastric cancer patients treated with irinotecan (70 mg/m^2, Days 1 and 15) and cisplatin (80 mg/m^2, Day 1). Expression of p53, bcl-2, VEGF was examined immunohistochemically in biopsy samples.

Results: The overall response rate and the median survival time were 55% (30/55) and 321 days, respectively. Thirty patients with intestinal-type adenocarcinoma survived longer than 25 patients with diffuse-type (median survival time: 446, 259 days, $P = 0.013$). The favorable phenotypes for chemoresponse were p53-negative, bcl-2-negative and VEGF-positive, which were in accordance with previous findings. The response rate was significantly correlated with the total number of these favorable phenotypes ($P = 0.043$). The 39 patients having 2 or 3 favorable phenotypes (p53-negative, bcl-2-negative and VEGF-positive) survived longer than the remaining 16 patients (median survival time: 444, 259 days, $P = 0.021$). In the Cox model, the number of the favorable phenotypes showed a tendency to correlate with survival after adjustment for potentially prognostic factors such as histological type or performance status ($P = 0.070$).

Conclusions: Immunohistochemical examination of biological markers may be useful in predicting the clinical outcome of unresectable gastric cancer patients treated with irinotecan and cisplatin.

Key words: gastric cancer – chemotherapy – p53 – bcl-2 – VEGF

INTRODUCTION

Cisplatin is an active agent against gastric cancer (1), and several chemotherapy regimens including cisplatin have been reported to show high response rates (2–5). Irinotecan, which inhibits DNA topoisomerase I, is also active against various malignancies including gastric cancer (6). Marked synergism, lack of cross-resistance, different mechanisms of action and relatively different profiles of adverse reactions between irinotecan and cisplatin have encouraged the combination of these agents, and it has shown promising results against gastric and lung cancers. In a phase II study for metastatic gastric cancer, the response rate was 59% and the median survival time was 322 days in 29 patients who had not received previous chemotherapy (7). However, in recent phase III studies, regimens of combined chemotherapy including cisplatin have failed to demonstrate a survival benefit compared

For reprints and all correspondence: Fumio Nagashima, MD, Department of Clinical Oncology, Saitama Medical School, 38 Morohongo, Moroyamama, Iruma-gun, Saitama, 350-0495, Japan. E-mail: fnagashi@saitama-med.ac.jp

with the single agent 5-fluorouracil (5-FU) (8–10), which had been developed more than 40 years ago (11). The severe toxicity of these cisplatin-containing regimens seems to be one of the reasons for no survival benefit despite the higher response rates as compared with 5-FU alone. It appears that we need to select an optimal regimen for each patient by predicting the chemotherapeutic efficacy. Recently, remarkable advances in the basic research have led to the identification of many biological markers indicative of sensitivity to some antineoplastic agents, some of which have been proved to have clinical impact.

Previously we reported that immunohistochemical examination of biological markers [p53, bcl-2, glutathione *S*-transferase-π (GST-π), thymidylate synthase (TS) and vascular endothelial growth factor (VEGF)] in biopsy samples was useful method for predicting the effects of chemotherapy. Our report showed that VEGF-positive, TS-negative, p53-negative, GST-π-negative, bcl-2-negative were favorable phenotypes in terms of chemoresponse, and the number of favorable phenotypes was a good indicator of both response and survival in patients with unresectable gastric cancer treated with 5-FU and cisplatin (12). Mutant p53 and bcl-2 proteins protect cancer cells from apoptosis induced by many antineoplastic agents and confer cytotoxic drug resistance (13–15). GST-π is an enzyme that plays an important role in cellular detoxification, and increases in this enzyme have been associated with resistance to antineoplastic agents such as CDDP (16–18). Because drug delivery is important for the sensitivity of tumors to antineoplastic agents, VEGF may contribute to chemoresponse through the promotion of angiogenesis and/or vascular permeability (19,20). In the present study, we investigated the relationship between immunohistochemical expression of VEGF, p53, bcl-2, GST-π and effects of chemotherapy in patients with unresectable gastric cancer treated with irinotecan and cisplatin. TS was not examined because 5-FU was not included in this regimen.

PATIENTS AND METHODS

Study Population

A total of 55 gastric cancer patients treated with irinotecan and cisplatin were retrospectively included in this exploratory analysis; the study subjects included nine patients entered into a phase II study of combination chemotherapy (7), which is one of experimental arms of ongoing randomized phase III trial in Japan (JCOG 9912), and 46 patients consecutively selected to be suitable for combination chemotherapy using the same regimen in clinical practice at the National Cancer Center Hospital East and its affiliated institutions. All the patients fulfilled the recruitment criteria used in JCOG 9912 except (viii): in brief, (i) histological confirmation of gastric cancer; (ii) Eastern Clinical Oncology Group scale performance status (PS) of 2 or better; (iii) age of 75 years or younger; (iv) no previous chemotherapy; (v) adequate bone marrow, liver and renal function; (vi) no other active malignancy; (vii) no severe medical complication; and (viii) primary tumors from which it was possible to obtain a sufficient amount of cancerous tissue for examining biological markers before chemotherapy.

Treatment Schedule

The treatment schedule of the combination of irinotecan and cisplatin and dose modification were the same as in the phase II study (7), briefly, drip infusion of irinotecan (70 mg/m^2, day 1 and 15) and cisplatin (80 mg/m^2, day 1) with adequate hydration. This treatment was repeated every 4 weeks until disease progression, patient refusal or unacceptable adverse reactions.

Evaluation of the Effects of Chemotherapy

Responses to chemotherapy in measurable lesions were evaluated by the standard World Health Organization response criteria (21). For primary lesions, responses were evaluated according to the criteria proposed by the Japanese Research Society for Gastric Cancer (22) using either gastroscopy or barium gastrography. Overall response was defined as the sum of the number of complete and partial responses. All patients were followed for at least 1 year after the initiation of chemotherapy, and survival time was defined as the period from the date of initiation of chemotherapy to the date of death due to any cause or the date of last confirmation of survival.

Immunohistochemical Examination

Immunohistochemical staining was performed in the same way as in our previous study (12). All immunohistochemical examinations were performed on tissue sections of formalin-fixed and paraffin-embedded biopsy specimens from primary tumors. Serial 3 μm thick slices were cut, deparaffinized in xylene and dehydrated with a graded series of ethanol solutions, then immersed in methanol containing 0.3% H_2O_2 for 20 min to inhibit endogenous peroxidase activity. The sections stained for p53 and bcl-2 were heated to 95°C by microwave irradiation for 10 min in phosphate-buffered saline (PBS) and 10 mM citrate buffer, respectively. The sections stained for VEGF 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 PBS (blocking buffer) for 60 min at room temperature, all sections were incubated overnight at room temperature with the primary antibodies diluted in blocking buffer to the following concentrations: anti-p53 antibody (Nichirei, Tokyo, Japan), 1:20 000; anti-bcl-2 antibody (DAKO, Glostrup, Denmark), 1:40; anti-GST-π antibody (MBL, Nagoya, Japan), 1:24 000; anti-VEGF antibody (Santa Cruz Biochemistry, CA, USA), 1:500. The sections were washed with PBS and then incubated with biotinylated second antibody diluted to 1:200 for 1 h. After washing with PBS, the sections were incubated with ABC reagent (Vector Laboratories, CA, USA), and the color was developed in a reaction

mixture containing 2% 3-3′-diaminobenzidine and 0.3% hydrogen peroxide in Tris buffer. The sections were then counterstained with hematoxylin or methyl green. The two investigators, F.N. and N.B., who were blinded to clinical outcome, assessed immunohistochemical staining independently. The intensity of staining of p53 and GST-π was graded as (++) when strong, as (+) when faint and as (−) when no staining was visible. For bcl-2, the intensity of staining was graded as (++) when stronger than that in the case of lymphocytes, as (+) when equal and as (−) when weaker than that in the case of lymphocytes. The staining of VEGF was graded as (+) when the intensity of staining in the case of the cancer cells was stronger than that in the case of stromal cells, as (±) when equal and as (−) when weaker. For all markers, cases were defined as positive when >20% of all cancer cells in each section showed (++) or (+).

Statistical Analysis

Chi-squared test was applied for comparisons between the expression of biological markers and the chemoresponse. Mantel test was applied for comparisons between the chemoresponse and the number of favorable phenotypes. Survival curve was constructed using Kaplan–Meier method and compared using log-rank test. Prognostic importance of the number of favorable phenotypes was analysed using the Cox regression model, which included tumor extension, histological and macroscopic tumor type, performance status, and age as covariates. These covariates were selected because they were recognized as important variables to predict survival in the previous study (12). Statistic analysis was performed by JMP ver. 4.0.5J software (SAS Institute, Inc., Cary, NC, USA).

RESULTS

Patient Backgrounds and Chemotherapeutic Effects

Clinicopathological features are listed in Table 1. The overall response rate was 55% (30/55), and the median survival time (MST) was 321 days. While the response rate did not differ between the patients with intestinal-type and diffuse-type (47%, 56%, $P = 0.843$), the former survived much longer than the latter (MST: 446, 259 days, $P = 0.013$).

Biological Marker Expression

Positive staining of p53 was observed in the nuclei of the cancer cells, whereas that of bcl-2 was observed in the cytoplasm. Staining of VEGF was observed in both cancer and stromal cells. The positive rates of p53, bcl-2, VEGF were 44% (24/55), 18% (10/55) and 64% (35/55), respectively. The clinicopathological features did not differ between the expression positive patients and the negative patients for p53, bcl-2 and VEGF, respectively. However, since GST-π-positive patients had a significantly better performance status than GST-π-negative patients, we excluded GST-π from the further investigation.

Table 1. Clinicopathological features of the subjects

Clinicopathological features	No. of patients (%)
Sex	
Male	37 (67)
Female	18 (33)
Performance status	
0	34 (62)
1, 2	21 (38)
Age (years)	
>60	27 (49)
≤60	28 (51)
Macroscopic type	
Non-scirrhous	20 (36)
Scirrhous	35 (64)
Histological type	
Intestinal	30 (55)
Diffuse	25 (45)
Degree of tumor extent	
Locally advanced	20 (36)
Metastatic	35 (64)

Table 2. Expression of biological markers and antitumor response

Marker	CR + PR (%)	NC + PD (%)	Total	*P*-value
p53 (−)	20 (65)	11 (35)	31	0.0915
p53 (+)	10 (42)	14 (58)	24	
bcl-2 (−)	25 (56)	20 (44)	45	0.9999
bcl-2 (+)	5 (50)	5 (50)	10	
VEGF (+)	22 (63)	13 (37)	35	0.1015
VEGF (−)	8 (40)	12 (60)	20	

CR, complete response; PR, partial response; NC, no change; PD, progressive disease.

Biological Marker Expression and Response

Higher response rates were observed in patients with p53 negative, bcl-2 negative and VEGF positive, respectively (Table 2). These relationships were in agreement with the previous findings (12). We, therefore, designated p53-negative, bcl-2-negative and VEGF-positive as favorable phenotypes for chemoresponse.

Biological Marker Expression and Survival

As a single factor, the patients with favorable phenotypes survived slightly longer than the patients without such

Table 3. Number of favorable phenotypes and antitumor response

No. of favorable phenotypes*	Antitumor response		
	CR + PR (%)	NC + PD (%)	Total
3	13 (72)	5 (28)	18
2	11 (52)	10 (48)	21
1 or 0	6 (38)	10 (62)	16

CR, complete response; PR, partial response; NC, no change; PD, progressive disease.
*Favorable phenotypes include p53-negative, bcl-2-negative and VEGF-positive.

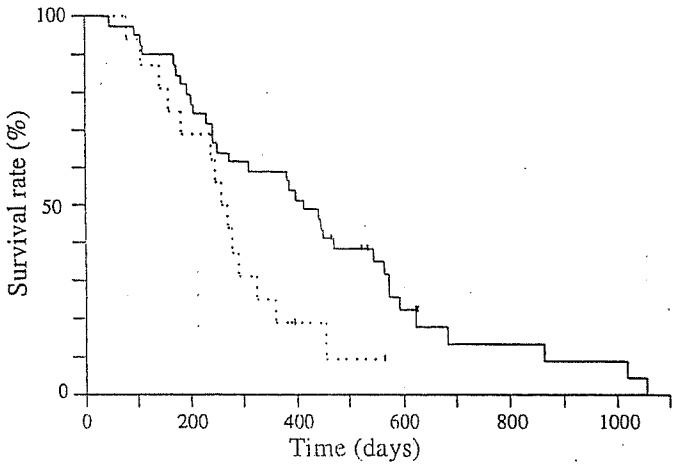

Figure 1. Survival of patients with or without favorable phenotypes. The solid line and dotted line represent patients with or without 2 or 3 favorable phenotypes, respectively ($P = 0.021$).

favorable phenotypes, but they were not significant (p53, $P = 0.504$; bcl-2, $P = 0.402$; VEGF, $P = 0.479$).

Combination of Biological Markers

The total number of these favorable phenotypes demonstrated a significant association with the response rate ($P = 0.043$, Table 3). Thirty-nine patients with 2 or 3 favorable phenotypes survived longer than the remaining 16 patients with statistical significance (MST: 444, 259 days, $P = 0.021$, Fig. 1). Table 4 shows relationships between the number of favorable phenotypes and clinicopathological features which were recognized as important prognostic factors in the previous study. Twenty-five (64%) of the 39 patients with 2 or 3 favorable phenotypes had intestinal-type adenocarcinoma histologically, whereas 5 (31%) of the 16 patients with 1 or 0 had intestinal-type ($P = 0.053$). There was no difference in other clinicopathological features between the two groups.

Multivariate Analysis

The covariates in the Cox model were set to the same as those in our previous study to improve the comparability between the previous and present results (Table 5). In the Cox model, the number of the favorable phenotypes showed a tendency to correlate with survival; the prognosis in patients having only <2 favorable phenotypes was poorer compared with that in patients having 2 or 3 favorable phenotypes (hazard ratio: 1.43, $P = 0.070$). Among the covariates in the model, histological type and performance status were significantly correlated with survival. Tumor extension, macroscopic type and age were not significant in the present study.

Table 4. Clinicopathological features and number of favorable phenotypes

Clinicopathological features	No. of favorable phenotypes		
	2 or 3 (%)	1 or 0 (%)	*P*-value
Age (years); median (range)	60 (26–74)	51 (26–68)	
Sex			
Male/female	26/13 (67/33)	11/5 (69/31)	0.9999
Performance status			
0/1, 2	23/16 (59/41)	11/5 (69/31)	0.7056
Macroscopic type			
Non-scirrhous/scirrhous	17/22 (44/56)	3/13 (19/81)	0.1238
Histological type			
Intestinal/diffuse	25/14 (64/36)	5/11 (31/69)	0.0537
Tumor extent			
Locally advanced/metastatic	13/26 (33/66)	7/9 (44/56)	0.2501

DISCUSSION

Our results support the hypothesis that immunohistochemical examination of biological markers may be useful in predicting the clinical outcome for unresectable gastric cancer patients receiving chemotherapy. The number of favorable phenotypes (p53-negative, bcl-2-negative and VEGF-positive) indicates chemotherapeutic effects. We are aware of no published reports that describe relation between biological markers and therapeutic effects in gastric cancer patients treated with irinotecan and cisplatin. Our results also confirm the results of the previous report that suggested the utility of combination of biological markers (12).

Up to the present, a few biological mechanisms have been implicated in determining the sensitivity to antineoplastic agents. Yeh et al. (23) reported that overexpression of p53 was not associated with resistance of gastric cancer to 5-FU-based systemic chemotherapy, whereas Nakata et al. (24) reported that p53 protein overexpression could serve as a predictor of the response to chemotherapy in gastric cancer. Thus, the correlation between some biological markers and chemoresponse is still controversial in cases of gastric cancer.

In the present study, patients who are either p53-negative, bcl-2-negative or VEGF-positive showed only a slightly higher response rate than the others. A single biological marker seems to have a small impact in predicting chemosensitivity, as shown in our previous study. Nakata et al. (25) investigated the relationship between bcl-2 and bax proteins and effect

Table 5. Cox proportional regression analysis for survival

Variable	Categories	*P*-value	Hazard rate ratio (95% CI)
No. of favorable phenotypes	2–3 versus 0–1	0.0704	1.433 (0.969–2.104)
Histological type	Intestinal versus diffuse	0.0066	1.695 (1.159–2.487)
Tumor extension	Locally advanced versus metastatic	0.1029	1.346 (0.941–1.942)
Performance status	0 versus 1 and 2	0.0208	1.514 (1.065–2.173)
Macroscopic type	Non-scirrhous versus scirrhous	0.6078	0.914 (0.651–1.293)
Age (years)	≤60 versus >60	0.7098	0.942 (0.684–1.287)

CI, confidence interval.

of chemotherapy in gastric cancer patients, and reported that among the bax-positive cases patients with bcl-2-positive tumors were significantly more resistant to 5-FU and had a worse prognosis than bcl-2-negative cases. Some other reports have also described the utility of combination of a couple of biological markers (26,27).

In the present study, the number of favorable phenotypes showed a clear correlation with response rates. Moreover, patients with 2 or 3 favorable phenotypes survived significantly longer than those with 1 or 0. From these results, the number of favorable phenotypes may be a good predictor of therapeutic effects in gastric cancer patients treated with irinotecan and cisplatin. Patients with intestinal-type adenocarcinoma survived longer than those with the diffuse-type, though the reasons for this difference are not clear. Yonemura et al. (28) reported a close relationship between VEGF-C expression, lymphatic spread and prognosis after surgery in gastric cancer patients. During the last few years, it was revealed that VEGF-A plays a role of prime importance in angiogenesis (29,30). The majority of subtypes of VEGF may explain the difference in chemoresponse.

The median survival time of the phase II study of irinotecan and cisplatin was 322 days, and other phase III studies for patients with metastatic gastric cancer generally showed median survival time of 7–11 months (8–10,31). And the overall response rate and survival in the present study were very similar to the results of phase II study of irinotecan and cisplatin. From these points, the overall survival time of patients with intestinal-type and 2 or 3 favorable phenotypes was remarkably long (data was not shown). Although survival elongation dose not always need high response rate, we already reported usefulness of biological markers and we have a tendency that the number of favorable phenotypes correlates to survival in the present study.

Investigating biological markers with consecutive patients eligible for enrollment criteria based on JCOG9912, as the present study is retrospective and some selection biases may not be excluded, we cannot confirm a utility of combinations of biological markers. We think a utility of biological markers should be confirmed in a large-scale study prospectively. The Japan Clinical Oncology Group had already initiated a three-arm randomized trial comparing 5-FU alone with S-1 alone and with irinotecan and cisplatin (JCOG9912). We are planning to investigate biological markers in this phase III trial.

Acknowledgments

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GASTROENTEROLOGY

Stool decay-accelerating factor as a marker for monitoring the disease activity during leukocyte apheresis therapy in patients with refractory ulcerative colitis

HIROYUKI KOHNO,* MOTOWO MIZUNO,* JUNICHIROU NASU,† CHIHO MAKIDONO,* SAKIKO HIRAOKA,* TOMOKI INABA,* KAZUHIDE YAMAMOTO,* HIROYUKI OKADA,* TEIZO FUJITA‡ AND YASUSHI SHIRATORI*

**Department of Medicine and Medical Science (Medicine 1), Okayama University Graduate School of Medicine and Dentistry, Okayama, †Department of Internal Medicine, National Shikoku Cancer Center, Matsuyama and ‡Department of Biochemistry, Fukushima Medical College, Fukushima, Japan*

Abstract

Background and Aims: We have shown previously that concentrations of stool decay-accelerating factor (DAF; CD55), a complement regulatory protein, in patients with ulcerative colitis (UC) are increased in relation to the severity of the colonic mucosal inflammation. In the present study, we evaluated the usefulness of stool DAF as a marker for monitoring disease activity in patients with steroid-resistant active UC being treated with leukocyte apheresis performed with a centrifugal cell separator.

Methods: Twenty-one patients with active and steroid-resistant UC were treated with leukocyte apheresis once a week for 4 weeks, and stool DAF concentrations were determined weekly by immunoassay.

Results: After treatment, 11 (52%) of the 21 UC patients went into remission. Stool DAF concentrations decreased promptly and steadily in the responsive group. The difference reached statistical significance as soon as after the second apheresis session ($P < 0.003$), compared with values before the therapy and corresponding values in the non-responsive group ($P = 0.024$). The reduction in stool DAF concentrations after the second apheresis session was significantly greater in the responsive group (median 90%, range 22–90%) than in the non-responsive group (median −13%, range −307–94%) ($P = 0.008$). Hematological tests, that is, white blood cell (WBC) count and C-reactive protein, declined significantly during the apheresis therapy, but not in relation to therapeutic response.

Conclusion: Stool DAF concentration is a useful marker in the clinical response of UC patients to treatment with leukocyte apheresis.

Key words: CD55, decay-accelerating-factor, leukocyte apheresis, ulcerative colitis.

INTRODUCTION

In the treatment of active ulcerative colitis (UC), leukocyte apheresis has been reported to be effective.[1–6] Apheresis is believed to exert its beneficial effects by removing activated leukocytes, including granulocytes and lymphocytes, and modulating altered immune responses. The effectiveness of apheresis is comparable to steroid therapy, even in patients whose colitis is refractory to conventional drug therapy.[4–6]

Assessment of the activity of intestinal inflammation is essential in the treatment of active UC. Laboratory markers such as white blood cell (WBC) count, platelet count, erythrocyte sedimentation rate and the acute phase protein, C-reactive protein (CRP), are useful in the evaluation of intestinal inflammation, but are not specific enough because often they are affected by drugs such as glucocorticoid or sulfasalazine. In the treatment with leukocyte apheresis, some of these markers, such as WBC and platelet counts, are decreased by apheresis itself and thus do not necessarily reflect the degree of intestinal inflammation.

We have previously shown that expression of decay-accelerating factor (DAF; CD55), a membrane-bound

Correspondence: Dr Motowo Mizuno, Department of Internal Medicine, Hiroshima City Hospital, 7-33 Motomachi, Nakaku, Hiroshima 730-8518, Japan. Email: mmizuno@isis.ocn.ne.jp

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glycoprotein which inhibits the formation and promotes the catabolism of C3 and C5 convertases,[7] is enhanced in the colonic epithelial cells of UC mucosa[8] and that stool DAF concentrations in UC patients are increased in relation to the severity of mucosal inflammation.[9] These observations suggest that measurement of stool DAF could be a useful non-invasive method of monitoring intestinal disease activity in patients with UC. To test this possibility, we measured DAF concentrations in serially obtained stool specimens from patients with steroid-resistant active UC during treatment with leukocyte apheresis, using a centrifugal cell separator.

METHODS

Patients and study design

Twenty-one patients with active, steroid-resistant UC (13 females and eight males, mean age 35 years, age range 14–69 years) were studied. The diagnosis of UC was based on history, clinical symptoms, and endoscopic and histological findings. Nineteen patients had pancolitis and two had left-sided colitis. Disease activity was graded on the basis of clinical features and laboratory data according to the criteria of Truelove and Witts,[10,11] and endoscopic findings according to the method described by Matts.[12] All patients had been refractory to corticosteroid therapy for 1 month or more (mean dose of predonisolone, 30 mg/day; mean duration of therapy, 6.1 weeks). Clinical disease activity of the patients was severe ($n = 8$) and moderate ($n = 14$). Endoscopic findings were: Matts grade 4 ($n = 7$), 3 ($n = 10$) and 2 ($n = 4$).

Leukocyte apheresis was performed by use of a centrifugal separation apparatus (Multi Component System, Haemonetics, Braintree, MA, USA) once a week for 4 weeks. In each session, leukocyte-rich fractions of buffy coat layers were removed from 2000 to 2500 mL of patients' peripheral blood. Acid citrate dextrose solution was used as the anticoagulant. All medications the patients were currently taking, including aminosalicylates and corticosteorids, were continued at the same dose during the study period. After the completion of four apheresis sessions, the patients' disease activity was again assessed. Remission was defined as a reduction in the degree of clinical activity to mild and without hematochezia or better, and improvement of endoscopic findings to Matts grade 1 or 2 without contact bleeding. Disease activity and remission were determined by two gastroenterologists (MM and HO) who had no knowledge of the stool DAF levels of patients under evaluation. Hematological parameters, WBC count, platelet count and CRP were monitored once a week, and spontaneously passed stool samples (1–5 g) were obtained from each patient before and weekly after each apheresis session until the end of the study. The study was conducted according to the guidelines of the Declaration of Helsinki and our local Ethics Committee approved the study protocol. The objective of this study was explained to each patient before the study, and written informed consent was obtained from each patient.

Determination of decay-accelerating factor in stool specimens

Stool specimens were weighted and suspended in three times the weight of phosphate-buffered saline containing 1% bovine serum albumin, 0.05% Tween 20, and 1 mmol/L phenylmethylsulfonylfluoride with increased NaCl concentration (0.4 M) to reduce non-specific reactions as described.[13] The suspensions were centrifuged at 8000 g for a few seconds in a benchtop microfuge[14] and supernatants were collected and kept frozen at –80°C until use. Samples were coded, and the person doing the DAF assay had no knowledge of their origin.

Details of our methods for the measurement of stool DAF have been described.[9,13–16] Briefly, human DAF was purified from pooled human erythrocyte stroma, and mouse monoclonal antibodies to DAF were prepared.[17] Two of these mouse monoclonal antibodies (IgG1), clones 1C6 and 4F11, were used. The 1C6 antibody is directed to the active site on the DAF molecule, that is, short consensus repeat (SCR) 3, and the 4F11 antibody recognizes SCR 4.[18] The 1C6 monoclonal antibody was labeled with horseradish peroxidase as described.[19] The wells of microtiter plates were coated with 4F11 monoclonal anti-DAF antibody, and serially diluted stool supernatants were added to the wells. After washing, peroxidase-labeled 1C6 anti-DAF antibody was added. After further washing, bound 1C6 antibody was detected with 2,2′-azino-di-3-ethylbenzothiazoline-6-sulfonic acid as substrate. Optical densities at 415 nm were measured on an automated ELISA plate reader. A calibration curve was obtained from several dilutions of known quantities of purified DAF, and the concentrations of stool DAF were calculated. Samples were analyzed in duplicate and the results presented as ng/g stool.

Statistical analysis

For statistical analysis, the Mann–Whitney *U*-test, chi-squared test and Wilcoxon's signed rank test were used. Correlation was assessed using Spearman's rank correlation.

RESULTS

After leukocyte apheresis treatment, 11 (52%) of the 21 UC patients had gone into remission. No adverse event due to the treatment was observed. Clinical background characteristics, including disease activity level, were not different between the patients who responded to apheresis and those who did not (Table 1). Inflammatory markers were not significantly different between the two patient groups, except for slightly higher WBC counts in the non-responsive group ($P = 0.02$, Mann–Whitney *U*-test).

Stool DAF concentrations were determined once a week during apheresis therapy (Fig. 1). Before therapy, concentrations in the responsive group (38–2571 ng/g; median 226 ng/g) were not significantly different from

Table 1 Background characteristics of patients

	Responsive group ($n = 11$)	Non-responsive group ($n = 10$)
Age (years)†	34.9 ± 15.7	35.8 ± 17.8
Sex (male/female)	4/7	4/6
Disease activity‡ (severe/moderate/mild)	3/7/1	5/5/0
Endoscopic grade (Matts grade, 4/3/2)	4/6/1	3/4/3
Total/left colitis	9/2	10/0
Duration of disease (years)†	3.4 ± 3.9	4.8 ± 5.2
Daily dose of PSL (mg)†	31.0 ± 16.4	30.2 ± 28.5
WBC (μL)§	8400 (5400–18 400)*	13 950 (7400–25 000)
CRP (mg/dL)§	0.7 (0.0–33.9)	1.7 (0.1–1 6.5)
PLT ($\times 10^4$/μL)§	30.3 (19.5–57.8)	36.1 (17.4–78.7)
Stool DAF (ng/g)§	226 (38–2571)	303 (63–1138)

*$P = 0.02$ (Mann–Whitney U-test); †mean ± SD; ‡the severity of the illness by Truelove and Witts criteria;[10,11] §median (range). CRP, C-reactive protein; DAF, decay-accelerating factor; PLT, platelet counts; PSL, prednisolone; WBC, white blood cell count.

those in the non-responsive group (63–1138 ng/g; median 303 ng/g) (Table 1, Fig. 1). In the responsive group, stool DAF concentrations decreased promptly and steadily, reaching statistical significance as early as after the second apheresis session ($P = 0.003$, Wilcoxon's signed rank test), and after the third ($P = 0.008$) and the fourth sessions ($P = 0.008$) when compared with values before the therapy. In contrast, stool DAF concentrations in the non-responsive group were not significantly reduced until after the fourth apheresis session. The percent reduction (median 90%, range 22% to 99%) in stool DAF concentration in the responsive group after the second apheresis treatment was significantly greater than the corresponding value in the non-responsive group (median −13%, range from −307% to 94%) ($P = 0.008$, Mann–Whitney U-test). Stool DAF concentrations in the responsive group were significantly lower than concentrations in the non-responsive group after the second ($P = 0.024$), the third ($P = 0.013$) and the fourth ($P = 0.009$) apheresis sessions.

The patients' clinical disease activity was also assessed once weekly during apheresis therapy (Fig. 2). In the responsive group, the clinical disease activity decreased gradually, reaching statistical significance after the second apheresis session ($P = 0.035$, Wilcoxon's signed rank test), and after the third ($P = 0.007$) and the fourth sessions ($P = 0.003$) when compared with the activity before the therapy. However, decreases in the clinical disease activity in the non-responsive group also reached statistical significance after the second and later apheresis sessions ($P = 0.046$). When we assessed the relationship between stool DAF concentrations and the patients' clinical disease activity, stool DAF concentrations correlated significantly with the clinical severity of UC ($r = 0.57$, $P < 0.0001$, Spearman's rank correlation) (Fig. 3).

In the hematological parameters of the two patient groups, significant decreases were observed in platelet counts ($P < 0.01$, Wilcoxon signed-rank test), CRP ($P < 0.05$) and WBC counts ($P < 0.05$) in the responsive patients, but similar decreases were observed also in the non-responsive patients.

DISCUSSION

In this study we serially measured stool DAF concentrations during leukocyte apheresis treatment in patients with UC refractory to corticosteroid therapy. The major finding was that measurement of stool DAF is useful as a simple, non-invasive means for evaluating the effectiveness of leukocyte apheresis therapy. The monitoring of stool DAF appears to be useful in predicting the effectiveness of apheresis therapy; when the therapy was ultimately effective, a decrease in stool DAF concentrations usually became evident as early as after the second apheresis session.

Apheresis of leukocytes from the circulation of UC patients is reportedly often effective even when the disease is refractory to conventional drug therapy.[4–6] Among several methods for leukocyte apheresis, we used a centrifugal cell separator, which has been shown to be effective in steroid-refractory UC.[6] A major advantage of leukocyte apheresis treatment is a lower frequency of adverse events. Reported side-effects of leukocyte apheresis are only minor, such as nausea or vomiting, which improve soon after the treatment,[5,6] as was the case in our study.

For the evaluation of disease activity and the efficacy of treatment in UC, symptoms often are non-specific and hematological tests such as WBC count and platelet count are decreased by apheresis itself. Indeed, these markers declined significantly during the apheresis therapy, but not in relation to the therapeutic response. Invasive examinations, such as radiography and colonoscopy, although useful and informative, may be a burden to patients when the disease is active. Measurement of stool DAF concentration, non-invasive means for monitoring the degree of intestinal inflammation, appears suitable for this purpose, especially in the treatment with leukocyte apheresis. Not

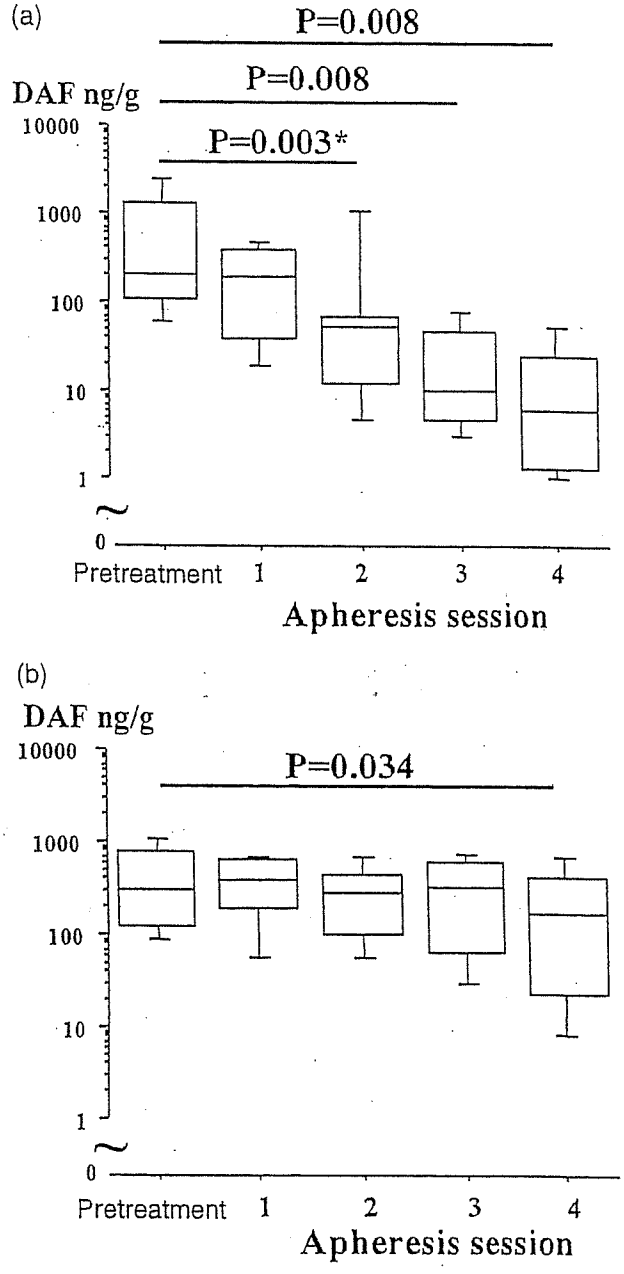

Figure 1 Weekly stool decay-accelerating factor (DAF) concentrations during apheresis treatment. (a) Apheresis responsive group; (b) apheresis non-responsive group. Dark bars represent median values, boxes represent interquartiles (the 25th and 75th percentiles), and error bars indicate the 10th and 90th percentiles. *Wilcoxon signed-rank test.

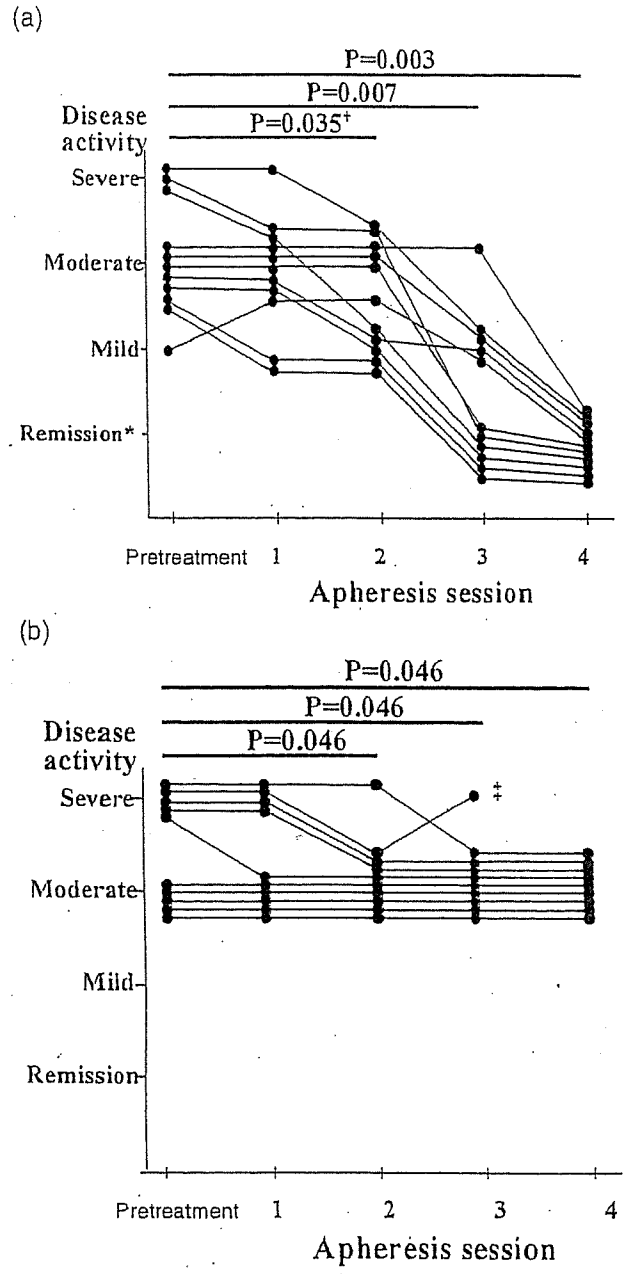

Figure 2 Weekly alterations of the disease activity during apheresis treatment. (a) Apheresis responsive group; (b) apheresis non-responsive group. The patients' disease activity was assessed according to the criteria of Truelove and Witts.[10,11] *Clinical activity of mild and without hematochezia or better. †Wilcoxon signed-rank test. ‡This patient was operated after the third apheresis session due to refractory bleeding.

only does the test accurately reflect disease activity,[9] it has been shown to be reliable and easy to perform.[13,14] DAF is resistant to proteolytic enzymes, such as trypsin,[20] so it is not affected by the abundant proteolytic enzymes derived from inflammatory cells that enter the colonic lumen during active UC. DAF is heat-resistant[21] and can be measured reliably in stools kept for at least 1 day at room temperature.[9] Other fecal proteins such as calprotectin,[22,23] a calcium-binding protein present in neutrophilic granulocytes,[24] and tumor necrosis factor-α,[25] have been proposed as surrogate markers of intestinal inflammation in inflammatory bowel disease. To our knowledge, these proteins have not been studied in the evaluation of leukocyte apheresis treatment.

DAF in stools possibly comes from several sources. We have shown that the colonic epithelia of active UC and colorectal cancer cells overexpress DAF on the

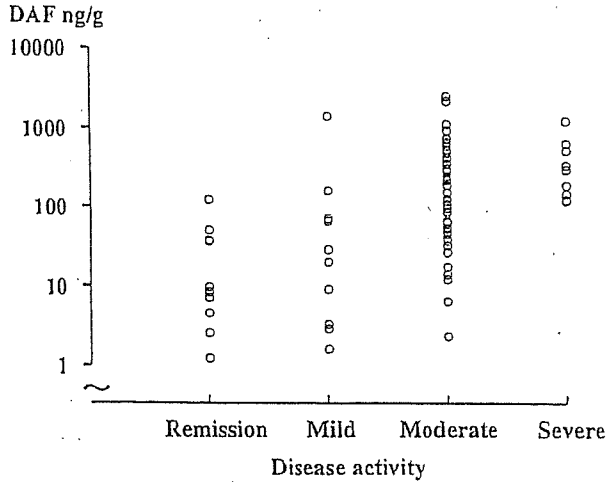

Figure 3 Stool decay-accelerating factor (DAF) concentration by patients' clinical disease activity in ulcerative colitis (UC). Stool DAF concentrations correlated significantly with the clinical severity of UC ($r = 0.57$, $P < 0.0001$, Spearman's rank correlation).

luminal surface[8,26] and that DAF is released into the colonic lumen from colorectal cancer cells by way of cleavage at the site of the membrane-bound anchor.[27] Thus, the first source is from colonic epithelial cells in the mucosal lesion and the second source of DAF may come from inflammatory cells passing into the colonic lumen. We have recently reported that serum concentrations and surface expression on WBC of DAF are increased in patients with active UC.[28] In active UC, inflammatory cells enter the colonic lumen through the diseased mucosa. Indeed, several proteins derived from neutrophils reportedly are increased in the stools of patients with active UC.[29,30] Plasma membranes of erythrocytes also are rich in DAF.[7] However, it is not likely that DAF in stools is derived from erythrocytes because we found no correlation between the amounts of DAF and hemoglobin in stools of UC patients in our previous study.[9]

We conclude that measurement of stool DAF concentrations may be useful in monitoring disease activity in patients with UC during leukocyte apheresis therapy, and as an early predictor of response to the therapy. A large-scale, prospective study of these possibilities is now warranted.

ACKNOWLEDGMENTS

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Conflict of interest statement: Dr Motowo Mizuno, Dr Teizo Fujita and Dr Takao Tsuji have patented the method for the detection of stool DAF.

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HOW I DO IT

CLINICAL APPLICATION OF AN INDWELLING NEEDLE FOR ESOPHAGEAL VARICES IN ENDOSCOPIC INJECTION SCLEROTHERAPY WITH SIMULTANEOUS LIGATION

YOSHIYUKI NISHIKAWA,* FUMIO CHIKAMORI,† MASATO MURAKAMI‡ AND JUNICHIROU NASU§

*Nishikawa Gastrointestinal Clinic and §Department of Internal Medicine, National Hospital Organization Shikoku Cancer Center, Matsuyama, †Department of Surgery, Kuniyoshi Hospital, Kochi and ‡Department of Internal Medicine, Murakami Memorial Hospital, Saijo, Japan

Endoscopic injection sclerotherapy (EIS), in which a sclerosing agent is infused into esophageal varices, is advantageous in obstructing a blood-supplying route; however, accurate intravascular infusion is technically difficult. To achieve accurate continuous intravascular infusion that does not depend on respiration or vomiting reflex, we developed an indwelling needle for esophageal varices. This indwelling needle facilitated intravascular puncture, as demonstrated for conventional puncture needles, and stabilized infusion of a sclerosing agent. Furthermore, EIS with ligation (EISL) prevented hemorrhage after the needle was removed. EISL with an indwelling needle may improve the treatment results.

Key words: endoscopic injection sclerotherapy (EIS), endoscopic injection sclerotherapy with ligation (EISL), esophageal varix, indwelling needle.

INTRODUCTION

A study of endoscopic injection sclerotherapy (EIS), an intravascular infusion procedure, has reported that infusion of a sclerosing agent into a blood-supplying route decreases the recurrence rate.[1,2] However, skilled techniques are required to puncture varices that move with respiration under an endoscope and continuously infuse a sclerosing agent using conventional puncture needles. Previous studies have used an indwelling puncture needle for accurate intravascular infusion; however, this procedure is not generally used. One reason is that when an indwelling puncture needle is used, the thickness of the barrel may increase the risk of hemorrhage with the removal of the needle. We performed simultaneous combination therapy with EIS and endoscopic variceal ligation (EVL) (modified EIS; i.e. EIS with ligation (EISL))[3–5] as standard treatment for esophageal varices. As the puncture site is ligated at the removal of a needle, EISL does not cause hemorrhage. Therefore, the risk of hemorrhage may be extremely low even when a thick indwelling needle is used. We report an indwelling needle for esophageal varices, the application of which has been facilitated by EISL.

MATERIALS AND METHODS

To prepare a taper-shaped indwelling needle for esophageal varices, the sheath end of a type C 23 G Barixer puncture needle for esophageal varices (Top Inc., Tokyo, Japan) was heated with a burner, extended, and cut at an appropriate point. The needle was sterilized with ethylene oxide gas before use. We used a GIF-Q240X scope (Olympus, Tokyo, Japan). EVL was performed using a pneumatic EVL device (Sumitomo Bakelite Co. Ltd, Tokyo, Japan).

For EISL with an indwelling needle for esophageal varices, an oral-side balloon was initially set at an area 1–2 cm from the end, and an EVL device was set at the scope end. For antisepsis, 100% ethanol was infused into the forceps pore. A scope was inserted to a varix, and the balloon was dilated. An indwelling puncture needle was inserted through the forceps pore to puncture the varix. After backflow of blood was confirmed, the inner cylinder was removed, and the barrel was inserted into the varix. Under direct vision, 5% ethanolamine oleate (EOI) was infused into the varix, involving a blood-supplying route. After the end of infusion, suction was performed during puncture. EVL involving the puncture site was performed (Fig. 1).

The indwelling needle that we produced has no adhesive area, and its shape was formed using heat. There is no difference in the basic structure between this needle and commercially available puncture needles, and the safety may be similar. An intensity test of the processed area was conducted to confirm the safety of this needle.

The present patient was treated in Kuniyoshi Hospital. The use of an indwelling needle has been approved by the Ethics Committee of this hospital. After explaining the procedure to the patient, written informed consent was obtained.

CASE REPORT

A 71-year-old female had an underlying disease of liver cirrhosis (type C). Endoscopy revealed an esophageal varix (F3, CB, Lm, RC(3+)),[6] and preventive treatment was performed.

Correspondence: Yoshiyuki Nishikawa, Nishikawa Gastrointestinal Clinic, 2-72 Katsuyama-cho, Matsuyama 790-0878, Japan.
Email: yonishik@ehime.med.or.jp

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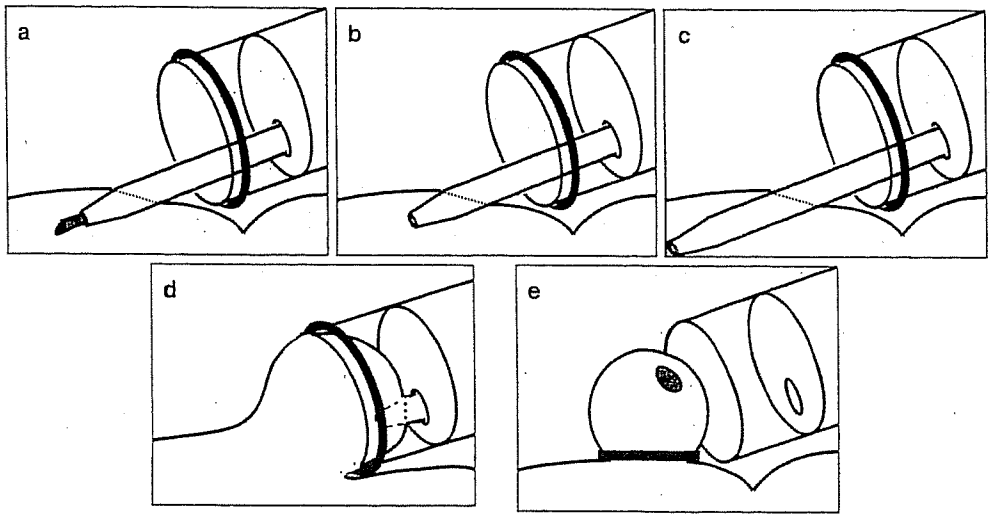

Fig. 1. (a) After puncture of the varix, backflow is confirmed. (b) The inner cylinder is removed. (c) The barrel is inserted, and 5% ethanolamine oleate is infused. (d) Suction is performed while pulling the puncture needle. (e) The lesion site involving the puncture site is ligated.

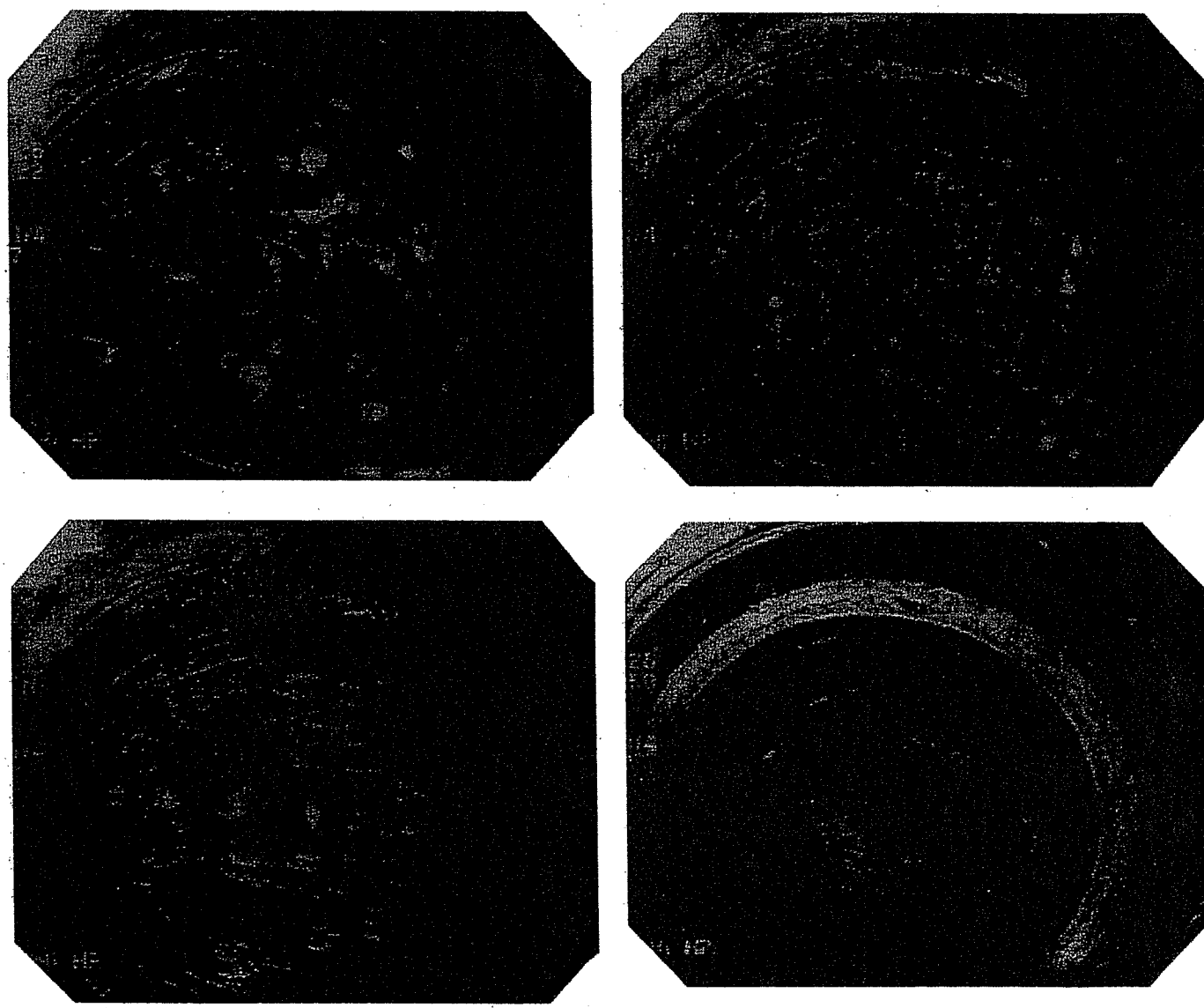

Fig. 2. (a) Before puncture. (b) After puncture, backflow is confirmed. (c) A sclerosing agent is infused via an indwelling needle. (d) After endoscopic variceal ligation there was no hemorrhage.

According to the Child-Pugh classification, the grade was evaluated as B. EISL was performed four times; in the first course, 10 mL EOI was infused into a blood vessel, and influx of EOI from the cardiac venous plexus to the left gastric vein was observed. In the second course, influx of EOI to the posterior gastric vein was observed after intravascular infusion of EOI at 10 mL. In the third course, influx of the sclerosing agent to the capillaries around the upper stomach was observed after intravascular infusion of EOI at 3 mL. In the fourth course, EVL alone was performed. After treatment, the endoscopic findings suggested FO and RC(–). There were no complications. This procedure stabilized intravascular infusion of the sclerosing agent (Fig. 2).

DISCUSSION

Endoscopic injection sclerotherapy,[7,8] in which intravascular infusion of a sclerosing agent is given, is a rational therapeutic procedure for obstructing a blood-supplying route. However, complete EIS; that is, infusion of a sclerosing agent into a blood-supplying route is often difficult in some patients.

Concerning EVL,[9–13] even less skilled therapists can perform complete treatment; therefore, the sufficient treatment response may be readily achieved, which leads to the underestimation of EIS in comparison to EVL. If the EIS procedure becomes simple and accurate, the therapeutic effects and assessment of EIS may improve.

We have developed a simple and accurate procedure so that therapists can perform complete EIS; namely, EISL, in which the puncture site is simultaneously ligated during EIS, and this indwelling needle for varices. The use of an indwelling needle for infusing a sclerosing agent eliminates the influence of respiration or vomiting reflex during infusion, facilitating accurate infusion of a sclerosing agent into a blood-supplying route, such as the left gastric vein. Conventional puncture needles were used by therapists for intravascular infusion, which depended on their skill. This requires high concentration. When an indwelling needle is used, relatively less skilled therapists can perform the procedure without stress, to a similar standard as skilled experts.

The reason why an indwelling needle has not commonly been used is the limitation of hemorrhage from the puncture site after the removal of the needle. The hole of an indwelling needle is larger than that of a standard puncture needle; therefore, hemorrhage from the puncture site may readily occur, and hemostasis may be difficult. To eliminate this limitation, EISL,[14] which we report as standard treatment for esophageal varices,[4] should be performed. In this procedure, the puncture site is ligated at the removal of the needle, reducing the risk of hemorrhage.

The use of an indwelling needle for esophageal varices may make EIS and EISL via intravascular infusion simpler and more accurate, emphasizing the significance of sclerotherapy for obstructing a blood-supplying route.

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Unusual Metastasis of Hepatocellular Carcinoma to the Esophagus

Eiji TSUBOUCHI, Shoji HIRASAKI, Junro KATAOKA, Satoshi HIDAKA, Takeshi KAJIWARA, Yuusuke YAMAUCHI, Toshikazu MASUMOTO and Ichinosuke HYODO

Abstract

This report describes a case of metastatic hepatocellular carcinoma (HCC) presenting as a polypoid mass in the lower esophagus. The patient was a 63-year-old man with HCC. An endoscopic examination revealed a pedunculated polypoid mass, about 3 cm in diameter, at the lower part of the esophagus. The biopsy specimen obtained from the tumor revealed that the mass consisted of a pseudoglandular arrangement of tumor cells, and the tumor was diagnosed as metastatic HCC. There were no symptoms due to esophageal tumor. He died of progressive hepatic failure. Cases of premortem-diagnosed esophageal metastasis from HCC are extremely rare.
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Key words: hepatocellular carcinoma, hematogenous metastasis, esophageal varix, gastric invasion, esophageal metastasis

Introduction

Metastasis to the esophagus is extremely rare, being present in less than 0.4% of patients with hepatocellular carcinoma (HCC) (1). This metastasis is presumed to be caused by tumor thrombi infiltrating via the portal system, at which point they are disseminated by hepatofugal portal blood flow to the gastrointestinal (GI) tract (2, 3). We present here a rare case of premortem-diagnosed esophageal metastasis from HCC.

Case Report

The patient was a 63-year-old man, 165 cm tall and weighing 52.3 kg. He visited our hospital for further evaluation of space-occupying lesions in the liver on April 23, 2002. He had a past medical history of HCC in 1998 at another institute. No specific family history was identified. At his first visit to our hospital, his body temperature was 36.8 °C, blood pressure was 94/50 mmHg, and radial pulse rate was 60 beats/min and regular. Moderate anemia was observed, but he did not have jaundice. A neurological examination revealed no abnormal findings and there was no lymphadenopathy.

Laboratory tests on April 23, 2002 showed a red blood cell count of $286\times10^4/\mu l$, a white blood cell count of 7,100/μl, and a platelet count of $11.0\times10^4/\mu l$. The hemoglobin concentration was 10.6 g/dl. The liver function tests revealed: aspartate aminotransferase, 61 IU/*l* (normal range [NR] 2–39); alanine aminotransferase, 43 IU/*l* (NR 2–39); total protein 6.1 g/dl (NR 6.5–8.1); serum albumin 2.1 g/dl (NR 3.8–5.2); alkaline phosphatase, 380 IU/*l* (NR 96–310); leucin amino peptidase, 100 IU/*l* (NR 45–71); γ-glutamyltranspeptidase, 22 IU/*l*; cholinesterase, 0.10 ΔpH (NR 0.6–1.2); lactate dehydrogenase, 298 IU/*l*; and total bilirubin, 2.1 mg/dl (NR 0.1–0.4). With respect to renal function, blood urea nitrogen was 13.6 mg/dl and creatinine was 0.8 mg/dl. Serological studies for hepatitis B virus were negative, however, hepatitis C virus was positive. A test for C reactive protein revealed a level of 0.10 mg/dl. Urinary protein and sugar were negative. Regarding tumor markers, carbohydrate antigen 19-9 was negative, however, carcinoembryonic antigen (CEA) and alpha fetoprotein (AFP) were high at 9.8 ng/ml (NR ≦5 ng/ml) and 4,130 ng/ml (NR ≦10 ng/ml), respectively. Abdominal CT on April 23, 2002 revealed a 3 cm low density area in the liver segment 8 treated at another institute with percutaneous ethanol injection and multiple space-occupying lesions (about 2 cm in diameter) in the liver in segment 2, 4, 5 and 6. The patient underwent intrahepatic arterial infusion (IAI) therapy with cisplatin (CDDP) 20 mg/m^2, day 1 and 5-fluorouracil 300 mg/m^2 days 1–5, repeated every week in May 2002. However, after 3 times of IAI, the

From the Department of Internal Medicine, Shikoku Cancer Center, Matsuyama
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Reprint requests should be addressed to Dr. Shoji Hirasaki, the Department of Internal Medicine, Shikoku Cancer Center, 13 Horino-uchi, Matsuyama 790-0007

Figure 1. Endoscopic appearance of the elevated lesion in the esophagus. The lesion presented as a polypoid mass, about 3 cm in diameter.

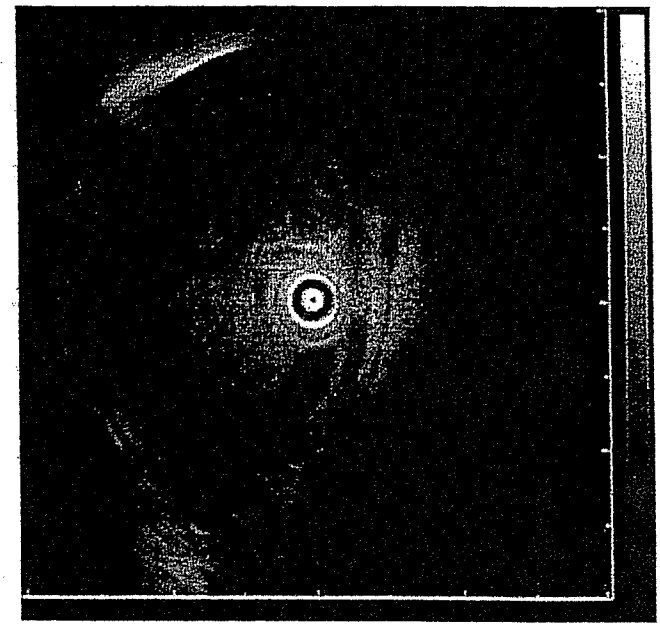

Figure 2. Endoscopic ultrasonography (EUS) revealed a polypoid mass with a mosaic echo pattern.

serum level of AFP had reached 9,005 ng/ml in June 2002. Thereafter, chemotherapy was stopped and he underwent palliative therapy.

Endoscopic examination of the upper digestive tract on January 17, 2003 revealed no abnormal findings except for F2 (enlarged and tortuous) esophageal varices. He underwent endoscopic examination again because of epigastric discomfort on April 17, 2003. It revealed a soft pedunculated polypoid mass, about 3 cm in diameter, at the lower part of the esophagus (Fig. 1). Esophageal varices were seen at the anal side of the tumor, however, it was unclear whether the tumor was connected to an esophageal varix. The tumor was slightly stained by lugol staining. Endoscopic ultrasonography (EUS) with a miniature probe of 20 MHz frequency using the water filling method revealed a polypoid mass with a mosaic pattern (Fig. 2). The arterial-dominant phase of dynamic CT showed enhancement of the esophageal tumor (Fig. 3). Abdominal CT on April 3, 2003 revealed space-occupying lesions in the liver in segment 2 and a direct invasion of HCC to the posterior gastric wall (Fig. 4). However, the existence of portal thrombus was not recognized on the abdominal CT. The biopsy specimen obtained from the esophageal lesion revealed that the tumor cells with pseudoglandular arrangement were covered with squamous cell epithelium (Fig. 5). They represented a positive immunohistochemical reaction for AFP. The pedunculated polypoid mass of the esophagus was diagnosed as a metastatic tumor from HCC. No therapy was done for the metastatic esophageal tumor or direct invasion of HCC to the posterior gastric wall because there were no symptoms due to the esophageal tumor, and GI bleeding did not occur at any time during his entire clinical course. Follow-up endoscopy on June 23, 2003 revealed that the metastatic tumor of the esophagus was slightly enlarged. The serum level of AFP had reached 596,090 ng/ml on July 9, 2003. He died of progressive hepatic failure on July 23, 2003. Autopsy was not permitted.

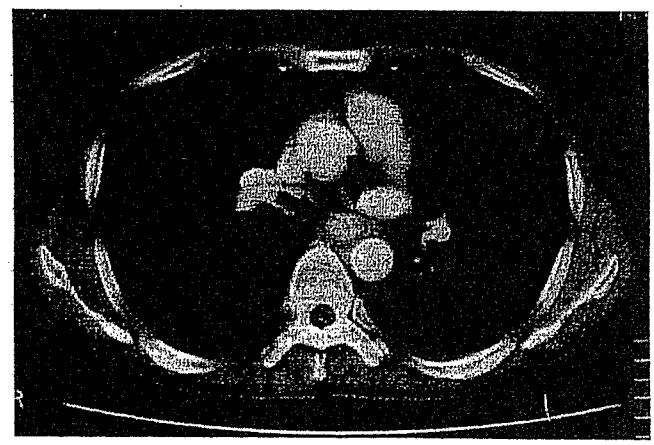

Figure 3. CT of the chest showed enhancement of the esophageal tumor (arrows).

Discussion

Autopsy and surgical series have suggested the presence of metastases of HCC in the lung (18.1–49.2%), lymph