Oncology: Principles and Practice. Philadelphia, PA: Lippincott Williams & Wilkins 1997; pp. 261–287.

7. Ajani JA, Ilson DH, Daugherty K et al. Activity of Taxol in patients with squamous cell carcinoma and adenocarcinoma of the esophagus. J Natl Cancer Inst 1994; 86: 1086–1091.
8. Adelstein DJ, Rice TW, Rybicki LA et al. Does paclitaxel improve the chemoradiotherapy of loco-regionally advanced esophageal cancer? A non-randomized comparison with fluorouracil-based therapy. J Clin Oncol 2000; 18: 2032–2039.
9. Conroy T, Etienne PL, Adenis A et al. Phase II trial of vinorelbine in metastatic squamous cell esophageal carcinoma. J Clin Oncol 1996; 14: 164–170.
10. Bleiberg H, Conroy T, Paillot B et al. Randomized phase II study of cisplatin and 5-FU versus cisplatin alone in advanced squamous cell esophageal cancer. Eur J Cancer 1997; 33: 1216–1220.
11. Iizuka T, Kakegawa T, Ide H et al. Phase II evaluation of cisplatin and 5-fluorouracil in advanced squamous cell carcinoma of the esophagus: a Japanese Esophageal Oncology Group Trial. Jpn J Clin Oncol 1992; 22: 172–176.
12. Hayashi K, Ando N, Watanabe H et al. Phase II evaluation of protracted infusion of cisplatin and 5-fluorouracil in advanced squamous cell carcinoma of the esophagus: a Japan Esophageal Oncology Group (JEOG) Trial (JCOG9407). Jpn J Clin Oncol 2001; 31: 419–423.
13. Ilson DH, Ajani J, Bhalla K et al. Phase II trial of paclitaxel, fluorouracil, and cisplatin in patients with advanced carcinoma of the esophagus. J Clin Oncol 1998; 16: 1826–1834.
14. Ilson DH, Saltz L, Enzinger P et al. Phase II trial of weekly irinotecan plus cisplatin in advanced esophageal cancer. J Clin Oncol 1999; 17: 3270–3275.
15. al-Sarraf M, Martz K, Herskovic A et al. Progress report of combined chemoradiotherapy versus radiotherapy alone in patients with esophageal cancer: an intergroup study. J Clin Oncol 1997; 15: 277–284.
16. Sparreboom A, van Tellingen O, Nooijen WJ, Beijnen JH. Preclinical pharmacokinetics of paclitaxel and docetaxel. Anticancer Drugs 1998; 9: 1–17.
17. Metges J, Hennequin C, Ychou M et al. Docetaxel as a second-line chemotherapy in metastatic esophageal cancer: a French study. Proc Am Soc Clin Oncol 2001; 20: 160a (Abstr 635).
18. Heath EI, Urba S, Marshall J et al. Phase II trial of docetaxel chemotherapy in patients with incurable adenocarcinoma of the esophagus. Invest New Drugs 2002; 20: 95–99.
19. Fujiwara K, Kohno I, Tanaka K et al. Phase II dose escalation: A novel approach to balancing efficacy and toxicity of anticancer agents. Anticancer Res 1999; 19: 639–644.
20. Therasse P, Arbuck SG, Eisenhauer EA et al. New guidelines to evaluate the response to treatment in solid tumors. J Natl Cancer Inst 2000; 92: 205–216.
21. National Cancer Institute. National Cancer Institute Common Toxicity Criteria. [On-line]. http: //ctep.info.nih.gov/reporting/index.html (2 April 2004, date last accessed).
22. Miller AB, Hogestraeten B, Staquet M et al. Reporting results of cancer treatment. Cancer 1981; 47: 207–214.
23. Kanehara S. Guidelines for the Clinical and Pathologic Studies on Carcinoma of the Esophagus, 9th edition. Tokyo, Japan: Japanese Society for Esophageal Diseases 1999.
24. Fleming TR. One-sample multiple testing procedure for phase II clinical trials. Biometrics 1982; 38: 143–151.
25. Einzig AI, Neuberg D, Remick SC et al. Phase II trial of docetaxel (Taxotere) in patients with adenocarcinoma of the upper gastrointestinal tract previously untreated with cytotoxic chemotherapy: the Eastern Cooperative Oncology Group (ECOG) results of the protocol E1293. Med Oncol 1996; 13: 87–93.
26. Taguchi T, Furue H, Niitani H et al. Phase I clinical trial of RP56976 (docetaxel) a new anticancer drug. Gan To Kagaku Ryoho 1994; 21: 1997–2005.
27. Extra JM, Rousseau F, Bruno R et al. Phase I and pharmacokinetic study of Taxotere (RP 56976; NSC 628503) given as a short intravenous infusion. Cancer Res 1993; 53: 1037–1042.
28. Tanigawara Y, Sasaki Y, Otsu T et al. Population pharmacokinetics of docetaxel in Japanese patients. Proc Am Soc Clin Oncol 1996; 15: 479 (Abstr 1518).

ELSEVIER

European Journal of Cancer 40 (2004) 1566–1571

European Journal of Cancer

www.ejconline.com

The ratio of thymidine phosphorylase to dihydropyrimidine dehydrogenase in tumour tissues of patients with metastatic gastric cancer is predictive of the clinical response to 5′-deoxy-5-fluorouridine

Tomohiro Nishina [a,*], Ichinosuke Hyodo [a], Jiro Miyaike [b], Tomoki Inaba [c], Seiyuu Suzuki [d], Yasushi Shiratori [e]

[a] *Department of Medical Oncology, National Shikoku Cancer Center, 13 Horinouchi, Matsuyama, Ehime 790 0007, Japan*
[b] *Department of Internal Medicine, Saiseikai Imabari Hospital, Japan*
[c] *Department of Internal Medicine, Kagawa Prefectural Central Hospital, Japan*
[d] *Department of Internal Medicine, Sumitomo Beshi Hospital, Japan*
[e] *Department of Medicine and Medical Science, Okayama University Graduate School of Medicine and Dentistry, Japan*

Received 6 October 2003; received in revised form 24 February 2004; accepted 25 February 2004
Available online 7 May 2004

Abstract

The aim of this work was to determine whether intratumour contents of thymidine phosphorylase (TP), which converts 5′-deoxy-5-fluorouridine (5′-DFUR) to 5-fluorouracil, and dihydropyrimidine dehydrogenase (DPD), which degrades 5-fluorouracil to inactive molecules, could be useful in predicting the response of patients with metastatic gastric cancer to chemotherapy using 5′-DFUR. Endoscopic biopsy specimens for the measurement of TP and DPD were obtained from the primary lesions before the start of combination chemotherapy, in which 5′-DFUR, cisplatin and mitomycin C were administered. TP and DPD were measured by enzyme-linked immunosorbent assays after the objective responses to chemotherapy had been confirmed. Twenty five patients were enrolled in this study and data for 22 patients in whom responses were confirmed were analysed. The median levels (ranges) of TP and DPD were 80 (4.9–360) and 44 (15–82) U/mg protein, respectively. The median value (range) of TP to DPD ratios was 1.9 (0.25–5.1). Eight patients with a complete or partial response to chemotherapy had significantly higher TP to DPD ratios than did the remaining patients with stable or progressive disease ($P = 0.014$). When a cut-off level of TP to DPD ratio was defined as the median value, the high-ratio group ($n = 11$) showed a significantly higher response rate (64% vs. 9.1%, $P = 0.024$) than the low-ratio group ($n = 11$). Overall survival of the high-ratio group was significantly longer than that of the low-ratio group (the median survival time; 300 days vs. 183 days, $P = 0.047$). The efficacy of 5′-DFUR could be optimised by preselecting patients with high TP/DPD ratios in their tumour tissues, and this would be applicable to the treatment with capecitabine.

Keywords: Thymidine phosphorylase; Dihydropyrimidine dehydrogenase; Gastric cancer; 5′-Deoxy-5-fluorouridine; Capecitabine; Mitomycin C; Cisplatin; ELISA

1. Introduction

Many methods for predicting the susceptibility of a cancer to various chemotherapy regimens have been investigated. One of the best known is chemosensitivity testing by culturing of tumour cells with the chemotherapeutic agents [1,2]. Another useful approach is the analysis of enzymes involved in the activation or inactivation of chemotherapeutic agents. However, the clinical relevance of such tests has not been established.

Thymidine phosphorylase (TP) is an enzyme involved in pyrimidine nucleoside metabolism. It has been

* Corresponding author. Tel.: +81-89-932-1111; fax: +81-89-931-2428.
E-mail address: tnishina@shikoku-cc.go.jp (T. Nishina).

0959-8049/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.ejca.2004.02.019

recently reported that TP is identical to platelet-derived endothelial cell growth factor and has been implicated in angiogenesis [3–5]. It has also been reported that high expression of TP in tumours was indicative of a poor prognosis [6]. TP is expressed in a wide variety of solid tumours (carcinomas of the breast, stomach, colon, pancreas and lung), and its content is higher in tumour tissues than in adjacent normal tissues [7,8]. Capecitabine and 5′-deoxy-5-fluorouridine (5′-DFUR), which is an intermetabolite of capecitabine, are oral prodrugs of 5-fluorouracil (5-FU), and TP is an essential enzyme that converts them to 5-FU [9,10]. High amounts of TP in tumours are suggested to enhance the efficacy of 5′-DFUR [11]. 5′-DFUR is currently being used for the treatment of gastric cancer in Japan [12,13]. Conversely, 5-FU is catabolised to biologically inactive molecules such as dihydrofluorouracil by dihydropyrimidine dehydrogenase (DPD) and DPD reduces the efficacy of 5-FU against tumours [14–16].

Recently, it was revealed that the efficacy of 5′-DFUR was correlated with the ratio of TP to DPD (TP/DPD) activity in human cancer xenograft models [17]. Furthermore, a clinical study using 5′-DFUR in adjuvant chemotherapy showed that patients with a high TP/DPD ratio in gastric cancer tissues had longer disease-free survival than did the patients with a low TP/DPD ratio [18]. Thus, the efficacy of 5′-DFUR is strongly influenced by the contents of TP and DPD in the tumour tissues. Recently, convenient enzyme-linked immunosorbent assays (ELISA) for measuring TP and DPD in human cancer tissues were developed [7,8]. The TP and DPD contents determined by each ELISA showed good correlation in clinical samples of tumour tissues with those determined by enzyme-activity assays [7,8]. This enabled us to measure those enzymes quantitatively in small portions of biopsy specimens, some comprising as little as 10 mg of tissue.

On the basis of these data, we conducted a prospective multicentre trial to investigate the relation between TP and DPD in tumour tissues measured by ELISA and the efficacy of 5′-DFUR.

2. Materials and methods

2.1. Patient population and eligibility

Patients were eligible for this study if they met the following inclusion criteria: (a) Patients having histologically proven gastric adenocarcinoma; (b) patients with advanced or recurrent gastric cancer with measurable metastatic lesions; (c) biopsy specimens taken from the primary lesion for the TP and DPD assays; (d) patients not less than 20 years old; (e) performance status of 0–2 (Eastern Cooperative Oncology Group); (f) no prior 5′-DFUR administration; (g) no severe major organ dysfunction; (h) no severe complications; (i) expected to survive more than 8 weeks and (j) written informed consent obtained.

The institutional review board of each participating institution approved this study.

2.2. Treatment methods

The treatment regimen was as follows: 5′-DFUR, 1200 mg/m^2 per day, was given orally from days 1 to 5; cisplatin, 10 mg/m^2 per day, was given by 30-min intravenous drip infusion on days 1 and 4; mitomycin C (MMC), 5 mg/m^2 per day, was given by bolus intravenous injection on day 8. This treatment was repeated every 2 weeks and was continued until progression or until occurrence of unacceptable adverse reactions.

2.3. Response criteria and treatment evaluation

The response to treatment was evaluated according to revised World Health Organisation criteria (Response Evaluation Criteria in Solid Tumours) every 4–6 weeks [19]. The response of each patient to the treatment was assessed by a group of extramural reviewers. All adverse reactions were graded according to the National Cancer Institute Common Toxicity Criteria version 2.

2.4. Preparation of biopsy specimens

Fresh endoscopic biopsy specimens (at least five biopsy samples from each patients) taken for the measurement of TP and DPD were sampled from primary lesions before the start of chemotherapy, after informed consent had been obtained. Samples from each patient were immediately frozen and stored at −80 °C. After the response to chemotherapy had been confirmed, each specimen was homogenised in a 10-fold volume of 10 mM Tris–HCl buffer (pH 7.4) containing 15 mM NaCl, 1.5 mM $MgCl_2$ and 50 μM potassium phosphate, then centrifuged at 10,000*g* for 15 min. The supernatant was stored at −80 °C. The protein concentration in the supernatant extracted from the tumour tissue was determined using a DC Protein Assay Kit (Bio-Rad Laboratories, Hercules, CA).

2.5. TP ELISA

TP in tumour tissues was measured by ELISA [7], and the enzyme contents were expressed as U/mg protein, where 1 U was equivalent to the amount of TP generating 1 μg of 5-FU in 1 h. The interassay precision of TP ELISA had a coefficient of variation (CV) of 8.6%.

2.6. DPD ELISA

DPD in tumour tissues was measured by a sandwich ELISA using two monoclonal antibodies specific to human DPD [8]. Enzyme contents were expressed as U/mg protein, where 1 U was equivalent to the amount of DPD catabolising 1 pmol of 5 FU/min. The interassay precision of DPD ELISA had a CV of 2.5%.

2.7. Statistical analysis

Differences among TP and DPD contents and TP/DPD ratios were analysed using the Mann–Whitney U test. Differences in response rates were analysed using Fisher's exact test. Overall survival rate was determined using the Kaplan–Meier method, and the log-rank test was used to calculate the difference in survival between the groups. P-values $\leqslant 0.05$ were regarded as statistically significant. All analyses were performed using SPSS software (version 11.5J; SPSS Inc., Tokyo).

3. Results

3.1. Case analysis and background factors

A total of 25 eligible patients was enrolled in the study between April 1999 and March 2002. Three patients could not have their responses evaluated because 5′-DFUR could not be administered sufficiently, due to gastrointestinal stenosis, and their treatments were terminated or changed too early. Responses to the chemotherapy were confirmed for 22 patients, and TP and DPD in the biopsy specimens from these patients were determined by ELISA. The backgrounds of these patients are shown in Table 1.

Table 1
Patient characteristics

Characteristics	Number of patients ($n = 22$)
Age (years)	
Median	66
Range	32–78
Sex	
Male	13
Female	9
Performance status (ECOG)	
0	10
1	10
2	2
Prior treatment	
Chemotherapy	3
Target lesion	
Lymph node	18
Liver	7
Lung	1
Histopathological type	
Differentiated	12
Undifferentiated	10

ECOG, Eastern Cooperative Oncology Group.

3.2. Response to chemotherapy and toxicity

The responses to treatment were: complete response (CR) one, partial response (PR) seven, stable disease (SD) six and progressive disease (PD) eight. The overall response rate was 36% (8/22). Grade 3 or 4 toxicity was caused by anorexia (4%), neutropenia (28%), anaemia (8%) and thrombocytopenia (12%). There were no treatment-related deaths.

3.3. Relationship between TP and DPD and responses to chemotherapy

The values of TP, DPD and TP/DPD ratios for each patient are plotted in Fig. 1(a), (b) and (c), respectively. The median TP for all 22 patients was 80 U/mg protein (range 4.9–360 U/mg protein) and that of DPD was 44 U/mg protein (range 15–82 U/mg protein). The median TP/DPD ratio was 1.9 (range 0.25–5.1). Neither TP nor DPD contents were significantly different between the responder (CR + PR) and the non-responder (SD + PD) groups ($P = 0.25$ and $P = 0.23$, respectively) (Fig. 1(a) and (b)). There was a considerable overlap for the distribution of TP/DPD ratios between responders and non-responders, but the ratios were significantly higher in the responder group than in the non-responder group ($P = 0.014$) (Fig. 1(c)). When a cut-off level for TP and DPD contents was assigned as the median value, there were no significant differences in response rates between the high- and low-level groups (response rate for TP, 55% vs. 18%, $P = 0.18$; response rate for DPD, 27% vs. 46%, $P = 0.66$). However, when the median value of the TP/DPD ratios was designated as a cut-off level, the high TP/DPD ratio group had a significantly higher response rate than did the low ratio group (64% vs. 9.1%, $P = 0.024$) (Table 2).

There was no significant correlation between the TP/DPD ratios and the severity of toxicities.

3.4. Survival

Only one patient was alive for 630 days up to the final follow-up time and the remaining patients were all dead due to tumour progression. The overall median survival time (MST) was 240 days. When each cut-off level for TP and DPD was assigned as the median value, there were no significant differences in survival between the high- and low-level groups (MST for TP, 207 days vs. 284 days, $P = 0.91$; MST for DPD, 240 days vs. 207 days, $P = 0.62$). When the median value

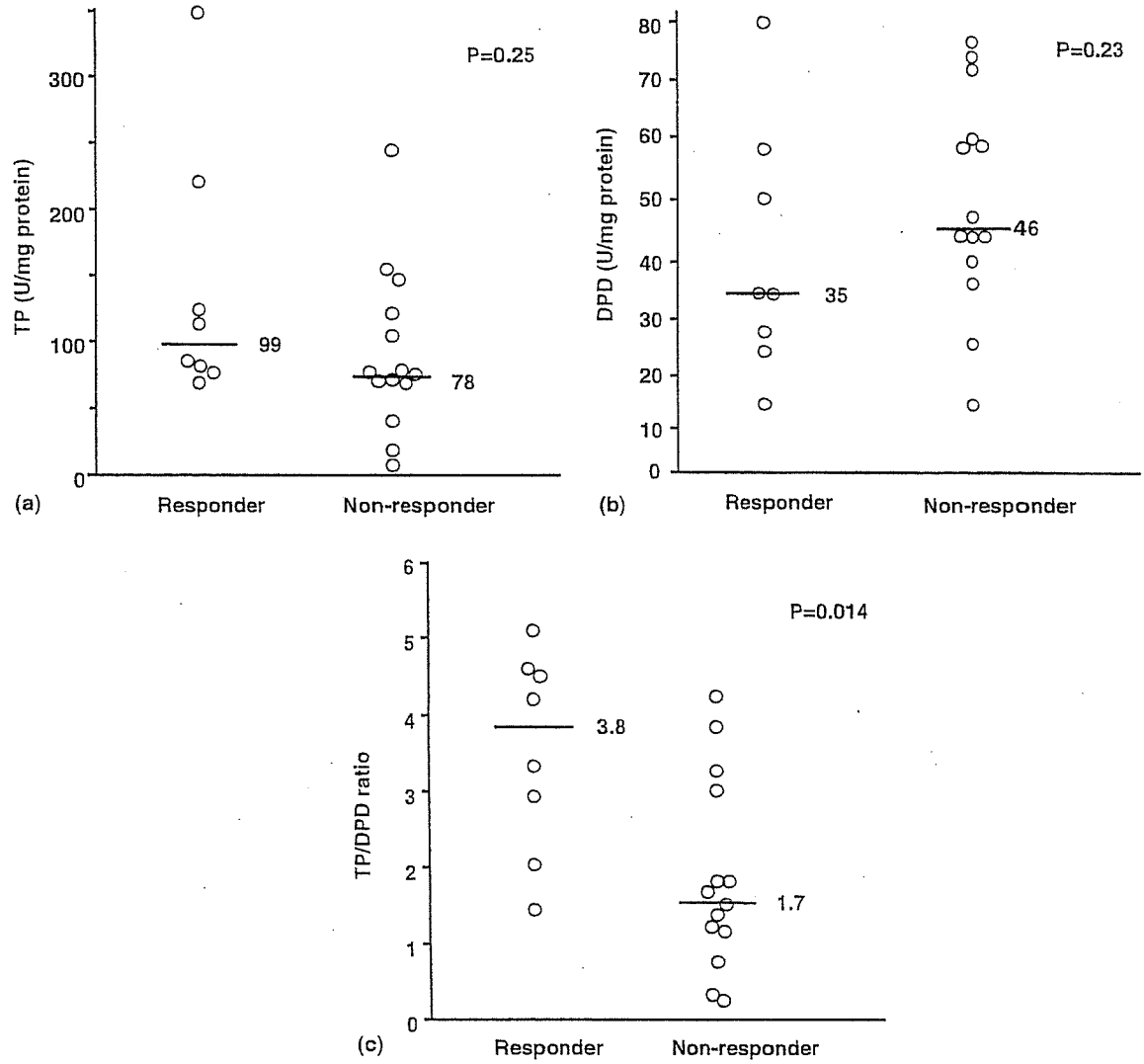

Fig. 1. Comparison of thymidine phosphorylase (TP) (a) and dihydropyrimidine dehydrogenase (DPD) (b) contents (U/mg protein) and TP/DPD (c) ratios in tumour tissues for the responder and non-responder groups. No significant difference between the groups was found for TP ($P = 0.25$, Mann–Whitney U test) and DPD ($P = 0.23$, Mann–Whitney U test) contents. The TP/DPD ratio was significantly higher in the responder group ($P = 0.014$, Mann–Whitney U test). Short horizontal line indicates the median value of each parameter in responder and non-responder groups.

of the TP/DPD ratios was designated as a cut-off level, the MST was 300 days for the high-ratio group and 183 days for the low-ratio group (Fig. 2). There was a marginal, but significant, difference between the two groups ($P = 0.047$).

4. Discussion

The biologically active drug 5-FU is formed selectively from 5′-DFUR by enzymatic conversion in TP-rich tumour tissues [10]. However, 5-FU is subsequently

Table 2
Response and TP/DPD ratios

TP/DPD	Response				Response rates (%)	95% CI
	CR	PR	SD	PD		
High ratio (⩾ 1.9)	1	6	3	1	64*	31–89
Low ratio (<1.9)	0	1	3	7	9.1*	0.2–41

CI, confidence interval.
CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease.
* $P = 0.024$ (Fisher's test).

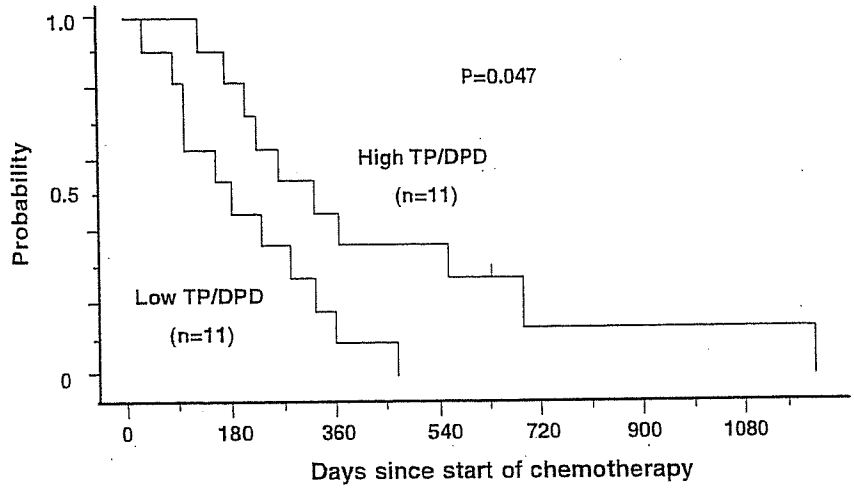

Fig. 2. Survival plots for patients with high and low thymidine phosphorylase/dihydropyrimidine dehydrogenase (TP/DPD) ratios in tumour tissues. Survival for the patients with high TP/DPD ratios was significantly longer than that for the patients with low ratios ($P = 0.047$, log-rank test).

catabolised to inactive molecules by DPD [14,15]. We showed a significant correlation between the ratios of these enzymes in tumour tissues and the clinical response to 5′-DFUR with improved survival in patients with metastatic gastric cancer, although the sample size was small.

In this study, although it was difficult to predict the response to 5′-DFUR based on either TP or DPD contents alone, the TP/DPD ratio in tumour tissues was a good predictor of tumour response to chemotherapy for metastatic gastric cancer. These data are consistent with the recent reports that the TP/DPD ratio in tumours was a better predictor of susceptibility to 5′-DFUR than either TP content or DPD content alone in human cancer xenograft models [17] and in a clinical study of adjuvant therapy for advanced gastric cancer [18].

There are many methods for the measurement of TP and DPD, including immunohistochemistry, a transcription-polymerase chain reaction method and an enzyme-activity assay [7,11,20]. The immunohistochemical method is the most widely used because of its convenience, but it is difficult to measure the amounts of TP and DPD quantitatively. In stead, we used ELISA to measure TP and DPD in this study. This method is also convenient and allows the quantification of enzyme activity in small pieces of tissue such as endoscopic biopsy specimens. These quantitative data allow the investigators to judge the results objectively. However, this method contains some weakness. All of the biopsy specimens taken may not be always tumour tissues, and intratumoural heterogeneity in TP content has been reported [21]. To avoid these biases in sampling, more than five biopsy specimens were obtained carefully from the same site of the primary lesion at which the diagnosis of adenocarcinoma had been confirmed by previous endoscopy. Another concern of this study is that TP and DPD contents of primary lesions were used as the predictor for the response, although the lesions that were used as indicator lesions for the response assessment were predominantly lymph nodes or liver metastases. To the best of our knowledge, there are no reports that TP/DPD ratios in primary tumour tissues correspond to those in metastatic lesions. Our data demonstrate that the measurement of TP and DPD enzyme contents in the primary tumour tissue could help in predicting the response to 5′ DFUR in metastatic disease sites.

Phase II studies using 5′-DFUR in Japan have shown a response rate of 14.3% (20/140) for patients with inoperable gastric cancer [12]. One promising approach for optimising therapy would be to combine 5′-DFUR with other agents, such as upregulators of TP and other biochemical modulators. Several anticancer drugs, including MMC, upregulate the expression of TP, and MMC in combination with 5′-DFUR shows synergistic activity in several human cancer xenograft models [22]. Cisplatin combination therapy affects biochemical modulation with 5-FU; cisplatin increased the availability of the reduced folate for tight binding of the 5-fluorodeoxyuridylate-generated form 5-FU to thymidylate synthase (TS) and enhanced the efficacy of 5-FU [23]. Therefore, in our present study, these drugs were combined to enhance the antitumour activity of 5′-DFUR. In fact, our study yielded a 36% response rate. This result was similar to those in several previous reports of 5′-DFUR combination therapies [13,24].

MMC upregulates the expression of TP in tumour tissues. Cisplatin reportedly has no effect on the expression of TP [22]. It is unclear how MMC or cisplatin influence the expression of DPD. Although we cannot exclude the possible influence of adding MMC and cisplatin on our finding that the TP/DPD ratio was predictive of response to 5′-DFUR, previous studies on the

expression of TP/DPD and the response to 5′-DFUR in experimental human xenograft models and in the clinical adjuvant chemotherapy setting strongly supports our conclusion [17,18]. Recently, capecitabine has been developed as a new fluoropyrimidine carbamate drug. It is a prodrug of the 5′-DFUR used in this study. The total amount of 5-FU generated from capecitabine within the tumours was 2.8- to 4.3-fold higher than that from 5′-DFUR [25]. High response rates have been reported recently for the treatment of gastric cancer with capecitabine [26,27]. Taking together with our findings, the response to capecitabine would be predicted more precisely by the use of TP/DPD ratio in the gastric cancer tissues. We are planning a clinical study to investigate the relative efficacies of capecitabine and the enzyme contents of TP and DPD, adding TS, which is the target of 5-FU and one of the predictors of its efficacy.

Acknowledgements

We thank Miss Noriko Amano for assistance with data collection. This work was supported in part by a Grant-in-Aid for Cancer Research (11S-3, 14S-3) from the Ministry of Health, Labor and Welfare, Japan. Some of this work was presented at the 2002 meeting of the American Society of Clinical Oncology.

References

1. Hamburger AW, Salmon SE. Primary bioassay of human tumor stem cells. *Science* 1977, **197**, 461–463.
2. Von Hoff DD. He's not going to talk about in vitro predictive assays again, is he? *J Natl Cancer Inst* 1990, **82**, 96–101.
3. Furukawa T, Yoshimura A, Sumizawa T, *et al.* Angiogenic factor. *Nature* 1992, **356**, 668.
4. Haraguchi M, Miyadera K, Uemura K, *et al.* Angiogenic activity of enzymes. *Natura* 1994, **368**, 198.
5. Moghaddam A, Zhang HT, Fan TP, *et al.* Thymidine phosphorylase is angiogenic and promotes tumor growth. *Proc Natl Acad Sci USA* 1995, **92**, 998–1002.
6. Takebayashi Y, Akiyama S, Akiba S, *et al.* Clinicopathologic and prognostic significance of an angiogenic factor, thymidine phosphorylase, in human colorectal carcinoma. *J Natl Cancer Inst* 1996, **88**, 1110–1117.
7. Nishida M, Hino A, Mori K, Matsumoto T, Yoshikubo T, Ishitsuka H. Preparation of anti-human thymidine phosphorylase monoclonal antibodies useful for detecting the enzyme levels in tumor tissues. *Biol Pharm Bull* 1996, **19**, 1407–1411.
8. Mori K, Hasegawa M, Nishida M, *et al.* Expression levels of thymidine phosphorylase and dihydropyrimidine dehydrogenase in various human tumor tissues. *Int J Oncol* 2000, **17**, 33–38.
9. Cook AF, Holman MJ, Kramer MJ, Trown PW. Fluorinated pyrimidine nucleosides. 3. Synthesis and antitumor activity of a series of 5′-deoxy-5-fluoropyrimidine nucleosides. *J Med Chem* 1979, **22**, 1330–1335.
10. Kono A, Hara Y, Sugata S, Karube Y, Matsushima Y, Ishitsuka H. Activation of 5′-deoxy-5-fluorouridine by thymidine phosphorylase in human tumors. *Chem Pharm Bull (Tokyo)* 1983, **31**, 175–178.
11. Koizumi W, Saigenji K, Nakamaru N, Okayasu I, Kurihara M. Prediction of response to 5′-deoxy-5-fluorouridine (5′-DFUR) in patients with inoperable advanced gastric cancer by immunostaining of thymidine phosphorylase/platelet-derived endothelial cell growth factor. *Oncology* 1999, **56**, 215–222.
12. Wakui A. Phase II study of 5′-deoxy-5-fluorouridine in the treatment of gastric cancer: a multicenter cooperative study. *J Int Med Res* 1988, **16**, 17B–18B.
13. Koizumi W, Kurihara M, Sasai T, *et al.* A phase II study of combination therapy with 5′-deoxy-5-fluorouridine and cisplatin in the treatment of advanced gastric cancer with primary foci. *Cancer* 1993, **72**, 658–662.
14. Naguib FN, el Kouni MH, Cha S. Enzymes of uracil catabolism in normal and neoplastic human tissues. *Cancer Res* 1985, **45**, 5405–5412.
15. Heggie GD, Sommadossi JP, Cross DS, Huster WJ, Diasio RB. Clinical pharmacokinetics of 5-fluorouracil and its metabolites in plasma, urine, and bile. *Cancer Res* 1987, **47**, 2203–2206.
16. Beck A, Etienne MC, Cheradame S, *et al.* A role for dihydropyrimidine dehydrogenase and thymidylate synthase in tumour sensitivity to fluorouracil. *Eur J Cancer* 1994, **30A**, 1517–1522.
17. Ishikawa T, Sekiguchi F, Fukase Y, Sawada N, Ishitsuka H. Positive correlation between the efficacy of capecitabine and doxifluridine and the ratio of thymidine phosphorylase to dihydropyrimidine dehydrogenase activities in tumors in human cancer xenografts. *Cancer Res* 1998, **58**, 685–690.
18. Terashima M, Fujiwara H, Takagane A, *et al.* Role of thymidine phosphorylase and dihydropyrimidine dehydrogenase in tumour progression and sensitivity to doxifluridine in gastric cancer patients. *Eur J Cancer* 2002, **38**, 2375–2381.
19. Therasse P, Arbuck SG, Eisenhauer EA, *et al.* New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. *J Natl Cancer Inst* 2000, **92**, 205–216.
20. Salonga D, Danenberg KD, Johnson M, *et al.* Colorectal tumors responding to 5-fluorouracil have low gene expression levels of dihydropyrimidine dehydrogenase, thymidylate synthase, and thymidine phosphorylase. *Clin Cancer Res* 2000, **6**, 1322–1327.
21. Kakeji Y, Maehara Y, Shibahara K, *et al.* Heterogeneity and clinical role of thymidine phosphorylase activity in gastric cancer. *Oncol Rep* 1999, **6**, 1213–1216.
22. Sawada N, Ishikawa T, Fukase Y, Nishida M, Yoshikubo T, Ishitsuka H. Induction of thymidine phosphorylase activity and enhancement of capecitabine efficacy by taxol/taxotere in human cancer xenografts. *Clin Cancer Res* 1998, **4**, 1013–1019.
23. Scanlon KJ, Newman EM, Lu Y, Priest DG. Biochemical basis for cisplatin and 5-fluorouracil synergism in human ovarian carcinoma cells. *Proc Natl Acad Sci USA* 1986, **83**, 8923–8925.
24. Koizumi W, Kurihara M, Hasegawa K, *et al.* Combination therapy with cisplatin, 5′-deoxy-5-fluorouridine (5′-DFUR) and mitomycin (MMC) in patients with inoperable, advanced gastirc cancer: a randomized trial comparing two dosage regimens. *Oncol Rep* 1996, **3**, 255–260.
25. Ishitsuka H. Capecitabine: preclinical pharmacology studies. *Investigat New Drugs* 2000, **18**, 343–354.
26. Koizumi W, Taguchi T. A phase II study of capecitabine (Xeloda™) in patients with advanced/metastatic gastric carcinoma. *Proc Am Soci Clin Oncol* 2001, **20**, 142b (Abstract 2320).
27. Kim TW, Kang YK, Ahn JH, *et al.* Phase II study of capecitabine plus cisplatin as first-line chemotherapy in advanced gastric cancer. *Ann Oncol* 2002, **13**, 1893–1898.

診療所編

ベストな選択をするために
4 地域医療の新たな展開

愛媛情報スーパーハイウェイと愛媛県医師会地域医療情報ネットワーク(EMAネット)

谷水正人(たにみず　まさひと)
国立病院四国がんセンター臨床研究部
佐伯光義,久野梧郎　松山市医師会
徳永昭夫,芳仲秀造　愛媛県医師会
木村映善　愛媛大学医学部付属病院医療情報部

はじめに

インターネット技術を利用した地域医療連携システムで,自立して実稼働しているものはまだ少ない。愛媛県医師会は,情報化の必要性を早くから(平成7年から)認識し,インターネットを活用してきた。愛媛県医師会地域医療情報ネットワーク(Ehime Medical Association Network:以下,EMAネット)は,平成15年現在ブロードバンドネットワーク化され,また,インターネットVPN(Virtual Private Network)に対応している。ブロードバンドネットワークとしてのEMAネットとそのサービスを紹介し,医師会におけるインターネット利用について考察したい。

EMAネットの立ち上げ

愛媛県医師会のEMAネットは,地域医療情報化と病診連携の推進を目的とし,平成7年にダイアルアップネットワークとしてスタートした[1),2)]。最初は,国立病院四国がんセンターを中継とする,がんネット(がん診療施設情報ネットワーク)の実験線として開始され,平成11年に独立した(128kbps専用線)[3)]。

しかし,ここ数年は,民間プロバイダーのブロードバンド常時接続と,ダイアルアップEMAネット接続の使い分けに,次第に困難を強いるようになっていた。折しも平成12年に,愛媛県行政から愛媛情報スーパーハイウェイ構想が提案された[4),5)]。愛媛県行政が,高速の閉域ネットワーク基盤を,行政,教育,産業,医療分野に提供しようという構想である。愛媛県医師会は県行政と議論の末,EMAネットと情報スーパーハイウェイ医療VPNを合体させることになった。また,ネットワークの拡大に合わせ,インターネットをブロードバンド化することになった。

ブロードバンドネットワーク化の目標

愛媛県の保健医療福祉ネットワーク構築を理想として,以下の目標を挙げた。

①県下すべての医療機関がEMAイントラネット常時接続できる基盤とする。
- 愛媛情報スーパーハイウェイ経由で救急指定病院(基幹病院)を常時接続させる。
- 中小病院と診療所向けに,インターネットVPN常時接続環境を構築する。

②医療情報提供と病診連携アプリケーションを拡充整備する。

③患者情報交換に対応できるシステムの安全性を確保し,運用体制を再構築する。

④ORCAプロジェクト推進の基盤とする。

システムの詳細

1. ブロードバンドネットワーク基盤

1) 情報スーパーハイウェイ(以下,愛媛情報SHW)とEMAネットの接続(図1)

愛媛情報SHWは,ATM回線(6～128Mbps,IP-VPN閉域専用線網)からなる。医療VPNには,県下の基幹病院,救急指定病院と保健所が常時接続される。県が企画運営する医療分野サービスは,愛媛県広域災害・救急医療情報システム,県立病院遠隔医療情報システム,デジタル検診画像遠隔診断システ

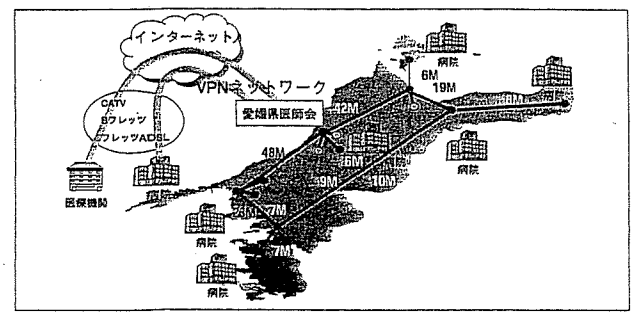

図1　愛媛県医師会ブロードバンドネットワーク(EMAネット)

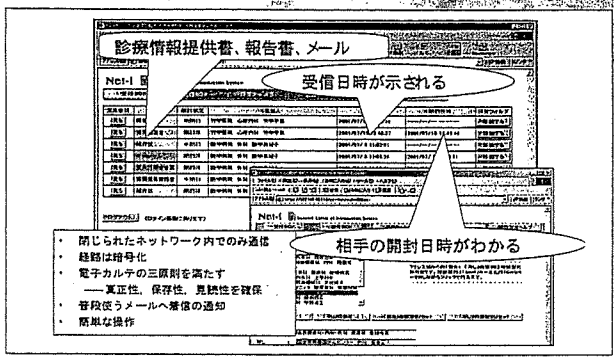

図2　Webmail紹介状

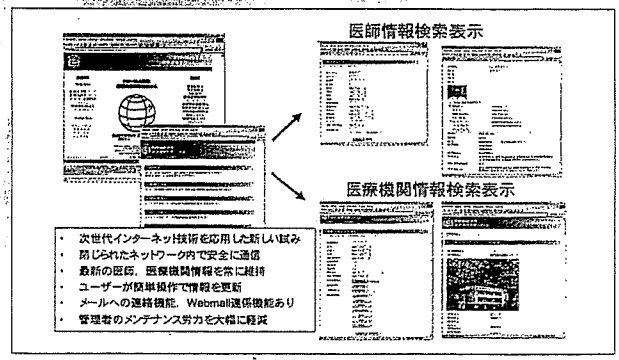

図3　P2P-Web連携病診連携支援システム

ム，精神科医療情報システム，成人病登録システムである。基幹線と施設は当初，ワイドLAN(NTT西日本10Mbps)，または，128Kbps専用線で接続された(インフラ整備状況に依存)。基幹医療機関側では，院内LANのルーティング制御で，EMAネット(ehime.med.or.jp)へのアクセスを愛媛情報SHW側に中継し，愛媛情報SHW内のDNS，プロキシサーバがEMAイントラネット側に中継する。

特筆すべきは，医療機関の公的役割が重視され，接続ポイントから各施設までの専用線敷設とその利用には，医療機関，医師会側の負担がない点である。また，将来の保健医療福祉ネットワークへの拡大を想定し，歯科医師会，薬剤師会，看護協会用にも施設数，会員数に見合うだけの十分なアドレス数を割り当てた。なお，接続ポイントから各施設までの間は，地域インフラの状況に合わせて定期的に見直され，光ファイバー，ADSL等を利用したフレッツオフィス型へ切り替えられている。

2) EMAネットのブロードバンド化とインターネットVPN対応

県医師会では，インターネットへの出口が，128Kbps専用線からケーブルインターネットへと変更された(640Kbps，その後8Mbps，平成16年2月から100Mbps，固定グローバルIP32個)。インターネットVPNとしては，ソフトウエアVPN〔Intel NetStructure VPN 3110と端末用ソフト(無料)〕，ハードウエアVPN(WatchGuard FireboxとWatchGuard SOHO間，ヤマハRT105e，RTX1000間によるルーター間VPN)が整備された。

末端ユーザーは，愛媛情報SHW経由のアクセスの場合，各医療機関のネットワーク管理者がサポートする。インターネットVPNによるアクセスの場合，ソフトウエアVPNは，ユーザーがEMAイントラネットからソフトウエアをダウンロードしてインストールする。ハードウエアVPNは，サポート業者が所定のルータを各医療機関の環境に合わせて個別に設定し，ネットワーク経由でサポートする。

2. EMAネットにおける医療情報提供と病診連携アプリケーション整備
——EMAネットのイントラネットのサービス

1) イントラホームページ情報提供とメーリングリスト

○EMAネットワーク情報の掲載：EMAネット解説，規約，設定書，接続申請書，接続ソフトのダウンロードなど

○医師会，医療関連の各種文書情報の掲載：日本医師会や厚生労働省からの通達文書，県医師会ニュース(理事会報告)，各種届出様式集，保険診療情報，感染症情報，愛媛県医師会員メールアドレス(病院医師のアドレス)掲載

また，EMAネット内郡市医師会のホームページでは，それぞれ独自のサービスが提供されている〔メーリングリストの運営，医師会FAX情報のメール配信，ハウジングサービス(市民向けメール配信サービス，グループウエア)など〕。

2) Webmail紹介状(図2)

これは，医療機関へのFAX患者紹介状のネットワーク版である。紹介状の交換や，紹介状の先送りが可能である。Web画面の空欄を埋めるだけで紹介状や返書が完成し，画像ファイルを添付できる。普段使うメールへも着信が通知され，相手の開封状況が送信者に表示される。EMAイントラネット内の通信であり，経路は暗号化されている。

3) P2P-Web連携病診連携支援システム(図3)

医師や医療機関の対応できる医療の詳細について，登録検索するシステムである。医療機関広告規制に容認された情報，病診連携に必要な情報〔在宅医療(リハビリ，緩和医療)，難病疾患への対応〕を掲載している。P2P(Peer to Peer)技術を用いており，登録内容はユーザー管理である。管理者はインデックスサーバのみを管理する[6]。

3. 患者情報交換に対応できるシステムの安全性と運用体制

EMAネットでは，安全確保のためファイアーウォール，ウイルススキャンを

谷水正人
（たにみず　まさひと）
国立病院四国がんセンター臨床研究部
1982年岡山大学医学部卒業。岡山大学医学部附属病院などを経て、93年から国立病院四国がんセンター内科に勤務。99年から同臨床検査部室長（医療情報担当）を併任。愛媛県医師会医療情報システム運営委員などを務める。

備え，サーバはRAID構成（1または5）とし，Webmail紹介状，病診連携支援システムへのアクセスは，IDパスワード，SSL通信を利用している。

EMAネットの運営は，県医師会総務部愛媛県医師会医療情報システム運営委員会が運用する。情報担当員2名（併任），保守技術員1名/1回/週を置いている。ネットワークシステム，アプリケーションについては，地元ベンダーと保守契約を結んでいる。

4. ORCAプロジェクト推進の基盤となるネットワーク

ORCAプロジェクト系は，EMAネット内でEMAイントラ系と内部的に切り分けている。平成14年12月に，ORCAプロジェクトコンソーシアムを立ち上げた。これはEMAネットとORCAプロジェクト系ネットワークを管理し，統一したセキュリティポリシーの下に，医療機関や関係ベンダー間の調整を図るためのものである。愛媛県医師会医療情報システム運営委員会，愛媛大学医学部医療情報部，ORCA協力業者（現在2企業），愛媛県医師会ネットワーク保守管理業者により構成される。

ブロードバンドネットワーク構築後の活動

新しいEMAネットは，平成14年5月から本格稼働した[3]。平成15年12月時点では，すべての郡市医師会と救急医療担当の基幹医療施設，診療所の120施設あまりが常時接続されている。現在は，ダイアルアップ利用を含めると，約485施設がEMAネットを利用している（愛媛県下の医療機関数は1300施設）。また，平成15年12月現在，県下の20施設で日医標準レセプトソフトが本稼働している。

考　察

医療は，本来公的な性格を持っており，医師会は行政と協力しつつ医療の情報化を主導し，安定した医療提供体制の確立と，患者サービスの向上を目指さなければならない。ブロードバンド対応したEMAネットは，県下のすべての医療機関に常時接続の機会を与え，病診連携ネットワークの基盤となった。それを活用して，医療情報，医師会情報の迅速な提供を中心に，会員へのネットワークサービスの充実を図りつつ，まずは医療現場の逼迫したニーズを反映させていく。それが一般県民への医療サービスの向上につながる。

EMAネットは，特定地域における患者情報共有型電子カルテには一歩引いている。構想自体が現場のニーズと乖離しており，地域医療全体を包含する医師会がかかわるには，まだ患者，医師のデジタルディバイドが大きすぎる。現在の医療経済情勢ではペイし得ないし，一度始めたら撤退は困難である。元来，クライアントサーバ型患者情報共有は，システムとして無理がある。現実に情報（患者カルテ）は，個々に（個々の医療機関に），バラバラに（対等に）存在しているものであり，必要に応じて個別の情報交換が行われている。これはP2Pネットワークに合致する。患者情報の共有は，地域医療を包含する立場の責任であるが，まだ次世代インターネットソリューションに期待するにとどめたい。

われわれにとって，愛媛情報SHWは，ブロードバンド化への大きな契機となった。しかし，今後ほかの地域に同じようなネットワーク形態が勧められるかと問われれば，答えは否である。さまざまな地域における情報SHWの意義は，すでに歴史的なものに変わりつつある。情報インフラは当初期待された以上に急速に進展した。必要なのは，それぞれの医師会，医療機関の内部の情報化とブロードバンド対応である。あとは，必要に応じてVPNなどで安全にイントラネットをつなげればよい。インターネット技術の進歩は急速であり，いま騒がれているVPNもいずれは不要になるであろう。方向はますます速く，安く，安全に，便利に，自由に，である。ブロードバンドと次世代インターネット技術が患者情報共有の課題も見事に解決してくれるに違いない。

●参考文献

1）秋山昌範：情報化社会における地域医療情報システムのあり方．第106回日本医学会シンポジウム記録集，59～66，1996.
2）野田益弘：愛媛県における医療情報システム―医師会情報システムを中心に―．日医ニュース，233・医療情報システム29，1998.
3）谷水正人：地域医療ネット―愛媛県医師会ネットワーク．カレントテラピー，20，1259～1265，2002.
4）愛媛情報スーパーハイウェイ（http://www.pref.ehime.jp/shw/index.htm）
5）ユーザー事例：自治体としての役割の中で地域と各分野の情報化を目指す情報スーパーハイウェイ．PACET，38～41，2001.（http://www.cisco.com/japanese/warp/public/3/jp/solution/casestudy/2001ehime/2001_ehime.html）
6）谷水正人・他：ブロードバンドネットワークによる常時接続型病診連携システム．医療情報学21回連合大会論文集，540～541，2001.

Efficacy of Clinical Pathway for the Management of Mucosal Gastric Carcinoma Treated with Endoscopic Submucosal Dissection Using an Insulated-tip Diathermic Knife

Shoji HIRASAKI, Masahito TANIMIZU, Toshikazu MORIWAKI, Ichinosuke HYODO, Toshiyuki SHINJI*, Norio KOIDE* and Yasushi SHIRATORI**

Abstract

Objective **Recently, the cases of early gastric carcinomas which can be treated by endoscopic submucosal dissection (ESD) method have been increasing in our institute. Simple and precise guidelines for treating mucosal gastric carcinoma are necessary for improving the treatment outcome of this disease. In our institute, ESD using an insulated-tip diathermic knife (IT-ESD) was introduced for the treatment of mucosal gastric carcinoma in 1996. The purpose of this study was to evaluate the impact of a clinical pathway and standardize the treatment for mucosal gastric carcinoma treated with IT-ESD.**

Materials and Methods **The Clinical Pathway and standardized of treatment for mucosal gastric carcinoma treated with IT-ESD were introduced at our institute in January 2002. We compared the length of hospitalization, total costs, hospital costs, operation time and bleeding rate during the 18 months before and after January 2002.**

Results **There was no significant difference in the clinical characteristics of the 20 patients in the control group and the 23 patients in the pathway group. There were 9 and 13 bleeding cases in the respective groups. The mean length of hospitalization, total costs and hospital costs were significantly less for patients in the pathway group. There was no significant difference in the operation time or bleeding rate among the two groups.**

Conclusion **Our clinical pathway and the standardization of treatment for mucosal gastric carcinoma treated with IT-ESD proved effective for treating patients with mucosal gastric carcinoma and for minimizing the length of hospitalization without compromising patient care.**

(Internal Medicine 43: 1120–1125, 2004)

Key words: gastric cancer, clinical pathway, endoscopic mucosal resection, complication

Introduction

The Clinical Pathway is a financial management system that was developed in the United States under the Diagnosis-Related Group (DRG) and the Prospective Payment Healthcare System (PPS). The Clinical Pathway originally was aimed to shorten the hospital stay and reduce healthcare costs, which has become an increasingly important issue in medicine. In Japan, it is also used for the standardization of medical care and to increase patient satisfaction with medical treatment.

Generally speaking, the Clinical Pathway and standardizing the treatment protocol for gastric carcinoma treated with endoscopic submucosal dissection using an insulated-tip diathermic knife (IT-ESD) is thought to be difficult to adopt because this disease shows various clinical patterns according to the size of tumor (1). The appearance of complications such as bleeding or perforation may further complicate the course of the disease (2), which will result in a longer hospital stay and high hospital costs in some cases. Recent advances in diagnostic techniques have enabled us to identify small and asymptomatic gastric carcinomas, and the cases of early gastric carcinomas that can be treated by endoscopic mucosal resection (EMR) have been increasing. Therefore, a

From Department of Internal Medicine, Shikoku Cancer Center, Matsuyama, *Department of Laboratory Medicine and **Department of Medicine and Medical Science, Okayama University Graduate School of Medicine and Dentistry, Okayama

Received for publication December 2, 2003; Accepted for publication June 27, 2004

Reprint requests should be addressed to Dr. Shoji Hirasaki, Shikoku Cancer Center, 13 Horino-uchi, Matsuyama 790-0007

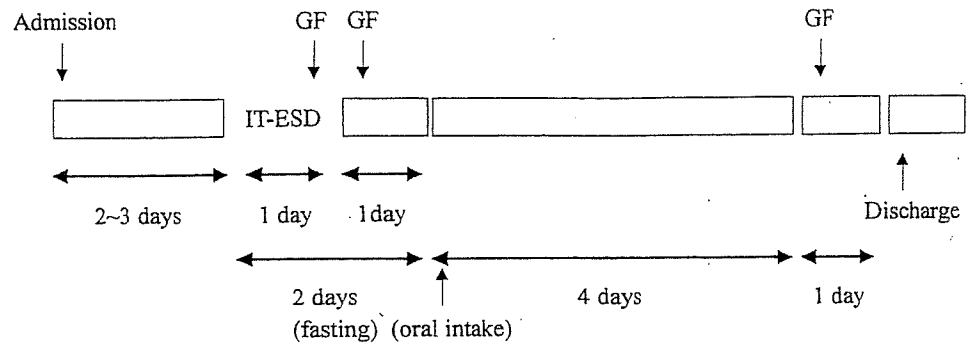

Figure 1. Schema of the clinical course. GF: gastrofiberscope, IT-ESD: endoscopic submucosal dissection using an insulated-tip diathermic knife.

simple and precise medical protocol with a clinical pathway is necessary to improve the treatment outcome and patient satisfaction. In our institute, IT-ESD was applied to patients with early gastric carcinomas up to 20 mm in diameter that were of a well or moderately histologically differentiated type, and were superficially elevated and/or depressed but without ulceration or definite signs of submucosal invasion. The purpose of this study was to determine the effect of the clinical pathway and standardization of treatment for mucosal gastric carcinoma treated with IT-ESD.

Materials and Methods

Preoperative assessment

Until recently, there was no standardized treatment protocol for gastric carcinoma treated with IT-ESD at our institute. Doctors made independent decisions about ordering endoscopic and radiological investigations, diagnosing of mucosal gastric carcinoma, and deciding on the indications for IT-ESD, timing of oral intake, timing of follow-up endoscopy and day of discharge. Until December 2001, patients who were going to undergo IT-ESD were examined by endoscopic ultrasonography (EUS) after admission. And the excision range was also determined after admission. Before the introduction of the clinical pathway, patients were discharged from hospital after meeting all of the following criteria: they could tolerate clear liquids and a regular diet; they had no symptoms due to IT-ESD including epigastric discomfort or abdominal pain; the lapse of 7 to 10 days after IT-ESD.

We retrospectively reviewed the records of patients admitted to Shikoku Cancer Center with mucosal gastric cancer between October 2000 and December 2001, just before the standardization of treatment with a clinical pathway for mucosal gastric cancer treated with IT-ESD was introduced to our hospital. Since January 2002, all patients admitted for mucosal gastric carcinoma who were going to undergo IT-ESD have been treated according to our treatment protocol (Fig. 1). The cases associated with other organ carcinoma were excluded from the study. The patients, who had severe underlying disease such as heart disease, respiratory disease, liver disease, or bleeding tendency, were excluded from the indication of pathway. The patients, who had drugs to promote bleeding such as ticlopidine, aspirin or warfarin, were indicated for this pathway after a definite term of discontinuance of drugs. There were no patients who had severe underlying disease or warfarin therapy in the control group and in the pathway group in this study.

All patients fulfilled the following criteria: 1) diagnosed as having mucosal gastric carcinoma by endoscopic findings or EUS, 2) had a biopsy specimen obtained from the lesion that revealed differentiated adenocarcinoma, 3) did not have an ulceration in the lesion, 4) had a tumor up to 20 mm in diameter. These conditions were confirmed by at least two doctors before IT-ESD. Since January 2002, patients who were going to undergo IT-ESD were examined by EUS before admission. The excision range was decided before admission. They were admitted to our hospital 2 or 3 days before IT-ESD (Fig. 1). Respiratory function and electrocardiogram were checked before IT-ESD. IT-ESD was performed under informed consent.

IT-ESD technique

IT-ESD was performed as we previously described (3): 1) marks were made at several points along the outline of the lesion with a coagulation current, using a marking tip (Type KD-1L; Olympus) (Fig. 2A), 2) an injection of 20 ml of saline containing 0.0025% epinephrine was carried out just outside the marks to prevent perforation until the mucosa around the lesion was raised. 3) a hole (about 2 mm) for inserting the ceramic ball of the IT-knife into the submucosal layer was made with hot biopsy forceps on the raised mucosa. 4) starting from the hole made by hot biopsy forceps, the mucosa just outside the marks with the IT-knife was incised. 5) after completion of the IT-knife cut around the lesion with a safe lateral margin (Fig. 2B), the submucosal tissue under the circumcised area was abraded with it (Fig. 2C), 6) As the abrasion made progress, the circum-

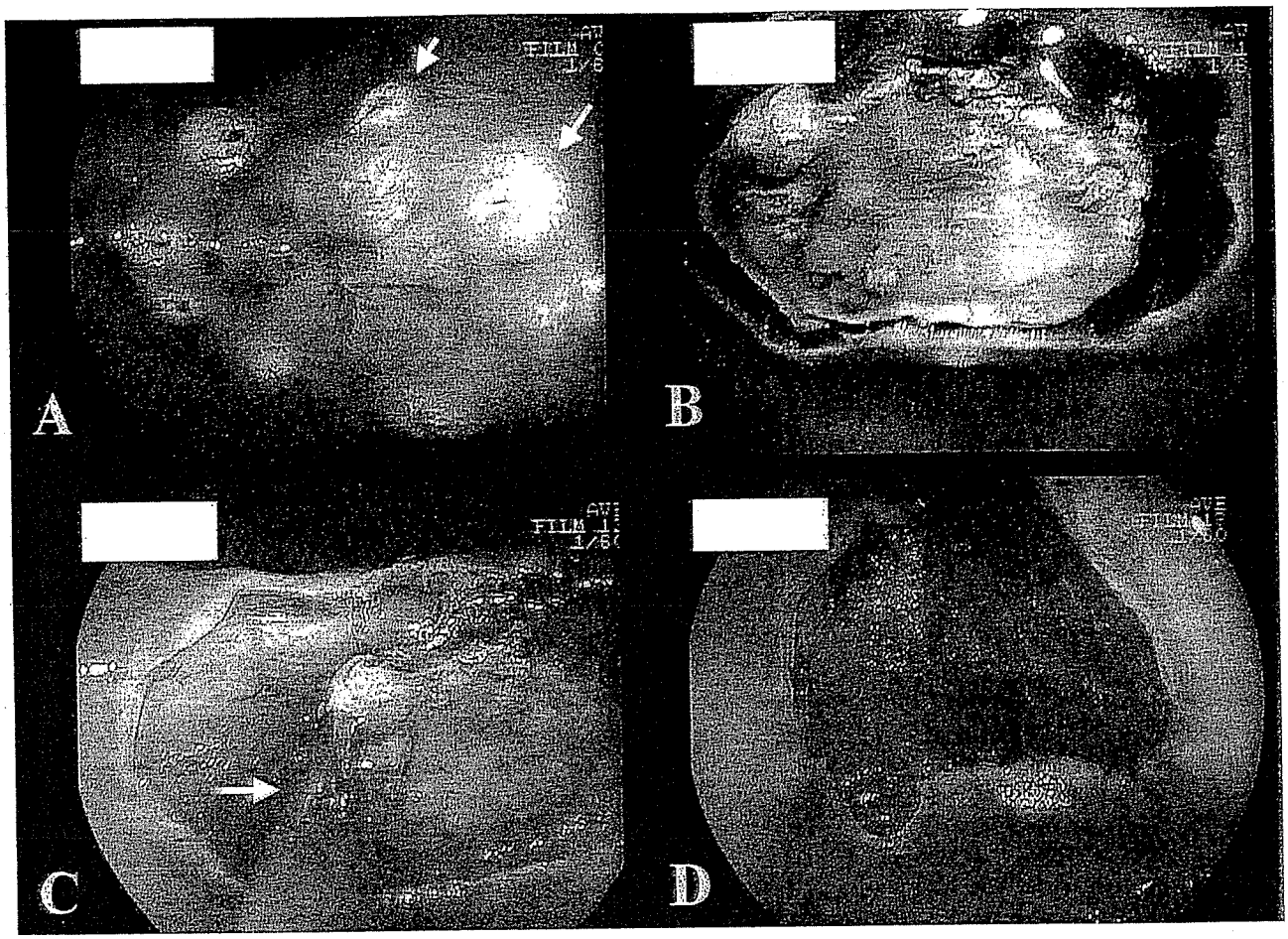

Figure 2. The procedure of IT-ESD; (A) marks (white arrows) are made at several points along the outline of the lesion with a coagulation current. (B) completion of the IT-knife cutting around the lesion with a safe lateral margin. (C) abrasion of the submucosal tissue under the circumcised area with IT-knife (white arrow). (D) the tumor is completely resected.

cised area shrank gradually, 7) an injection of saline with epinephrine was carried out to prevent perforation, until the circumcised area was sufficiently raised to snare the lesion, 8) mucosal resection was performed in the same way as with snare polypectomy (Fig. 2D).

Histological assessment

A gastrointestinal (GI) pathologist evaluated the EMR specimens with special attention to the depth of tumor invasion and the lateral and deep margins of excision. Resected specimens were cut into 2 mm slices according to the Japanese Classification of Gastric Carcinoma (4) and were evaluated histologically as to whether cancerous glands were present or absent at the margin of each slice.

Post EMR pathway

All patients were given intravenous proton pump inhibitor (PPI) postoperatively, followed by 4 weeks of oral PPI. Three to five hours after IT-ESD was finished, the first follow-up endoscopic examination was performed to determine whether bleeding would occur or not. The following morning, a second follow-up endoscopic examination was done and oral intake was started on the second postoperative day (Fig. 1). Blood tests were done to determine whether bleeding would occur or not on the next day of IT-ESD. Further examinations were done if necessary on a case-by-case basis. When the patient was free of bleeding or abdominal pain, a third follow-up endoscopic examination after IT-ESD was performed on the 6th postoperative day (Fig. 1). Patients were discharged from hospital after meeting all of the following criteria: they could tolerate clear liquids and a regular diet; a third endoscopic examination after IT-ESD revealed a decrease in the size of the postoperative ulcer. As a rule, the patients were discharged from hospital on the 7th postoperative day (Fig. 1).

Patients and methods

The outcome after introducing this clinical pathway and standardized treatment protocol was investigated. Clinical data were recorded from patients who were admitted to

Table 1. Characteristics of Patients and Lesions in the Control Group and the Pathway Group

	Control group	Pathway group	P value
Number of patients	20	23	
Mean age (years)	69.8±7.7	71.1±5.6	NS
Male: Female	15 : 5	17 : 6	NS
Size of the lesion (mm)	11.0±4.8	9.0±4.8	NS

NS: not significant.

Table 2. Results of Clinical Data of Patients in the Control Group and the Pathway Group

	Control group	Pathway group	P value
Total hospital days	17.5±6.9	10.9±1.9	<0.001
Preoperative hospital days (days)	7.2±5.2	1.9±0.3	<0.001
Postperative hospital days (days)	10.3±3.0	9.0±2.6	NS
Total costs (yen)	530,000±106,000	439,000±75,000	<0.01
Hospital costs (yen)	514,000±117,000	407,000±76,000	<0.001
Number of endoscopic examination	4.3±1.0	4.2±0.4	NS
Operation time (min)	56.6±34.7	48.4±25.7	NS
Size of the resected specimen (mm)	30.9±9.0	28.7±7.8	NS
Complications			
Rate of bleeding (%)	45	56	NS
Rate of perforation (%)	0	0	NS

NS: not significant.

Shikoku Cancer Center for mucosal gastric carcinoma between January 2002 and March 2003. The results were compared with the historical data of patients admitted to Shikoku Cancer Center for mucosal gastric carcinoma between October 2000 and December 2001, and were cared for in a different manner, not according to the pathway.

Patients in both periods were divided into a bleeding group and a non-bleeding group. The bleeding group was defined as patients who required endoscopic management by using methods such as clip placement and/or monopolar electrocoagulation to stop the bleeding. The total length of hospitalization, preoperative stay, postoperative stay, hospital costs, total costs (from the point of the diagnosis of gastric carcinoma to the point of discharge), the number of endoscopic examination (from the point of the diagnosis of gastric carcinoma to the point of discharge, excluding the point of IT-ESD), and operation times were compared among the groups. Patients, who were discharged from hospital on the 8th postoperative day or later, were judged as variance.

Statistics

Values are expressed as mean±SD. Statistical analysis was performed using the unpaired Student's t-test. A P value of less than 0.01 was considered to be significant.

Results

There were 21 patients in the control group and 24 in the pathway group. One patient in the control group and one patient in the pathway group were excluded from the study as they were associated with rectal cancer and underwent surgical treatment for rectal cancer after IT-ESD during the same hospitalization. All lesions in this study were resected as a single fragment. The diagnosis of mucosal gastric carcinoma was confirmed histopathologically in all patients who underwent IT-ESD.

The number of patients classified in each group and the clinical data of IT-ESD are shown in Table 1. There were no significant differences in any items tested between the two groups. The mean age of the 43 patients was 70.5±6.7 years (range, 48 to 84 years), being 69.8±7.7 years in the control group and 71.1±5.6 years in the pathway group. The size of the lesion was 9.9±4.8 mm (range, 2 to 19 mm), being 11.0±4.8 mm in the control group and 9.0±4.8 mm in the pathway group.

The data that should be compared between each group are shown in Table 2. There were no significant differences in the mean size of the resected specimen among the groups (30.9±9.0 in the control group versus 28.7±7.8 in the pathway group). The mean length of hospitalization became significantly shorter in the pathway group (17.5±6.9 in the control group versus 10.9±1.9 in the pathway group) ($p<0.001$). The mean preoperative stay was significantly

Table 3. Effect of Bleeding upon Total Hospital Days, Postoperative Hospital Days and Hospital Costs

	Control group			Pathway group		
	Bleeding	Non-bleeding		Bleeding	Non-bleeding	
Number of patients	9	11		13	10	
Size of the lesion (mm)	12.3±4.8	9.8±4.5	NS	9.2±4.6	8.6±4.7	NS
Size of the resected specimen (mm)	33.3±9.8	28.9±7.2	NS	31.2±6.1	25.4±8.6	NS
Total hospital days	18.7±8.1	16.5±5.5	NS	13.9±2.8	12.4±2.5	NS
Postperative hospital days	12.0±2.9	8.7±2.3	NS	10.2±2.7	8.0±0	NS
Hospital costs	533,000±126,000	392,000±133,000	NS	433,000±64,000	394,000±83,000	NS

shorter in the pathway group (7.2±5.2 in the control group versus 1.9±0.3 in the pathway group) ($p<0.001$). There were no significant differences in the mean postoperative stay among the groups (10.0±3.0 in the control group versus 9.0± 2.6 in the pathway group). The mean hospital cost in Japanese yen became significantly lower in the pathway group (514,000±117,000 in the control group versus 407,000±76,000 in the pathway group) ($p<0.001$). The mean total cost became significantly lower in the pathway group (530,000±106,000 in the control group versus 439,000± 75,000 in the pathway group) ($p<0.01$). There was no significant difference in the number of endoscopic examinations among the groups (4.3±1.0 in the control group versus 4.2± 0.4 in the pathway group). In the control group, all patients underwent endoscopic examinations at least 2 times namely, 1) at the point of diagnosis of gastric cancer and 2) on the day following IT-ESD. One patient underwent 6 endoscopic examinations: 1) at the point of diagnosis of gastric cancer, 2) at the point of EUS in admission, 3) five hours after IT-ESD 4) emergency endoscopy due to bleeding after IT-ESD in the night, 5) on the day following IT-ESD, and 6) on the 6th postoperative day. This was the case that underwent the most endoscopic examinations in the control group. In the pathway group, 19 of 23 patients underwent endoscopic examinations 4 times (once at the point of diagnosis of gastric cancer, 3 times according to treatment protocol). The remaining 4 patients underwent endoscopic examinations 5 times, one underwent an additional endoscopic examination due to bleeding after IT-ESD and 3 patients underwent an additional endoscopic examination due to EUS. The mean operation time of the 43 patients was 52.2±28.8 minutes (range, 15 to 120 minutes), being 56.6±34.7 minutes for the control group and 48.4±25.7 minutes (range, 15 to 120 minutes) for the pathway group. There were 22 (51.2%) cases of bleeding during or after IT-ESD: 9 (45.0%) in the control group and 13 (56.5%) in the pathway group. No patients required surgery, angiography or transfusion for bleeding. Perforation did not occur in any patient.

Effect of bleeding upon total hospital days, postoperative hospital days and hospital costs was shown in Table 3. There was no significant difference in the total hospital days, postoperative hospital days or hospital costs between the bleeding group and non-bleeding group in the control group and the pathway group (Table 3).

Five patients did not follow this pathway in the pathway group, and were discharged from the hospital on the 8th postoperative day or later, resulting in a variance rate of 21.7%. In all 5 cases, the start of diet was delayed due to bleeding.

Discussion

The Clinical Pathway has become widely adopted in Japan since its introduction by the DRG and PPS, and has been under trial in several hospitals since 1998 (5). In making a clinical pathway, the treatment of a particular disease needs to be standardized, but since this is a standard scale, some cases do not follow a predictable course and variance from the pathway is inevitable. Mucosal gastric carcinoma treated with IT-ESD shows various clinical patterns according to the size of tumor or the degree of bleeding, so variance from the pathway is a major problem that needs to be resolved.

Although the mean length of hospitalization was reduced after the introduction of this clinical pathway, there was no significant difference in the mean postoperative stay between the control group and the pathway group. The reduction of the mean length of hospitalization was due to shortening of the preoperative stay. This result has been realized due to the stipulation to confirm the depth of the tumor before admission. We were able to perform IT-ESD in a few days after admission because two or more endoscopists confirmed the depth of tumor or excision range before admission.

We classified cases of mucosal gastric carcinoma treated with IT-ESD into two groups, according to whether or not there was bleeding during IT-ESD or after. Because a bleeding case differs from a non-bleeding case in its endoscopic treatment and use of clips, it is associated with a longer postoperative stay and higher hospital costs. But there were no significant differences in the mean postoperative stay or hospital costs among the bleeding group and non-bleeding group. These results indicate that even patients with bleeding were treated without delay, although bleeding may complicate the course of administration. However, 5 patients did not follow this pathway, resulting in a variance rate of 21.7% in this study. In all 5 cases, the start of the diet was delayed

due to bleeding. We thought that the variance rate of 21.7% in this study was tolerated because nearly 80% of the patients followed the treatment protocol. It has been described that ordinarily about 20% patients do not follow the Clinical Pathway (6). The reported incidence of bleeding in IT-ESD was higher than in EMR using conventional methods. Ohkuwa et al (1) reported that the incidence of bleeding in IT-ESD was as high as 22%. In our study, the bleeding frequency was higher than Ohkuwa et al (1) reported. Thus, variant cases may decrease in the future, if the bleeding in IT-ESD is well controlled. Improved devices for the management of bleeding are certainly necessary to decrease the length of hospitalization. There were no perforation cases in this study. However, it is necessary to pay particular attention to perforations because Ohkuwa et al (1) and Ono et al (7) reported that the incidence of perforation with IT-ESD is 5%.

In the present study, operation time with IT-ESD was longer than that of EMR using conventional methods. Although the IT-ESD technique is complicated, it might be worth trying as the first therapy for mucosal gastric carcinoma because of its high one-piece resection rate (1, 2). The introduction of our treatment protocol did not affect the operation time of IT-ESD.

We decided that patients underwent 3 times of follow up endoscopy in the treatment protocol. Before introduction of the clinical pathway, all patients in the control group underwent a follow-up endoscopic examination the following morning. We create the first follow-up endoscopic examination to treat with bleeding soon after IT-ESD and the third follow-up endoscopic examination to confirm the improving of the postoperative ulcer. The result was that the total number of endoscopy in the pathway group did not increase compared with control group. We speculated that the creation of 3 times of follow up endoscopy in the treatment protocol did not affect the total costs or hospital costs.

We think that it is possible to hospitalize patients on the day before IT-ESD if the preoperative examination is finished as an outpatient. Consequently we can shorten preoperative hospital days and total hospital days. Now we are putting this idea into practice. In the pathway group, moreover, when bleeding did not occur at the second follow-up endoscopic examination, there was no GI bleeding thereafter. Thus, we are considering a new pathway in which the third follow-up endoscopic examination after IT-ESD is omitted and the patients are discharged from hospital on the 6th postoperative day.

By making the treatment protocol and criteria for discharge clear, patients and nurses were able to understand the treatment more clearly, which helped them to accept it. As a result, our clinical pathway and standardization of treatment for mucosal gastric carcinoma improved the satisfaction of both patients and nurses, being well accepted by both. Reducing hospital costs is of paramount importance because the number of cases of early gastric carcinomas that can be treated by EMR has been increasing at our institute every year. A simple and precise clinical pathway and medical guidelines for treating mucosal gastric carcinoma would have a great impact on hospitals, and promote patient satisfaction.

Our clinical pathway and standardization of treatment for IT-ESD for mucosal gastric carcinoma decreased the total length of hospitalization and overall healthcare costs. These results suggest that our clinical pathway and standardization of treatment for IT-ESD for mucosal gastric carcinoma had a successful outcome and promoted the quality of care.

References

1) Ohkuwa M, Hosokawa K, Boku N, Ohtu A, Tajiri H, Yoshida S. New endoscopic treatment for intramucosal gastric tumors using an insulated-tip diathermic knife. Endoscopy 33: 221–226, 2001.
2) Miyamoto S, Muto M, Hamamoto Y, et al. A new technique for endoscopic mucosal resection with an insulated-tip electrosurgical knife improves the completeness of resection of intramucosal gastric neoplasms. Gastrointest Endosc 55: 576–581, 2002.
3) Hirasaki S, Endo H, Nishina T, Masumoto T, Tanimizu M, Hyodo I. Gastric cancer concomitant with inflammatory fibroid polyp treated with endoscopic mucosal resection using an insulation-tip diathermic knife. Intern Med 42: 259–262, 2003.
4) Japanese Research Society for Gastric Cancer. Japanese classification of gastric carcinoma. Kanehara & Co., Ltd., Tokyo, 1999.
5) Takegami K, Kawaguchi Y, Nakayama H, Kubota Y, Nagawa H. Impact of a clinical pathway and standardization of treatment for acute appendicitis. Surg Today 33: 336–341, 2003.
6) Dykes PC. Desining and implementing clitical pathways: An overview. in: Dykes PC, Wheeler K (Eds). Planning, Implementing and Evaluating Clitical Pathways. Springer Publishing Company, New York, 1997.
7) Ono H, Kondo H, Gotoda T, et al. Endoscopic mucosal resection for treatment of early gastric caner. Gut 48: 225–229, 2001.

Seronegative Alpha-Fetoprotein-Producing Early Gastric Cancer Treated with Endoscopic Mucosal Resection and Additional Surgery

Shoji HIRASAKI, Masahito TANIMIZU, Takao TSUZUKI, Eiji TSUBOUCHI, Satoshi HIDAKA, Ichinosuke HYODO and Hisao TAJIRI*

Abstract

A 67-year-old man visited our hospital for the treatment of gastric carcinoma. Endoscopic mucosal resection was performed, however, histological examination of the resected specimen revealed tumor invasion to the submucosal layer with vessel invasion. Immunohistological studies were carried out on resected specimens and part of the cancerous lesion showed a positive reaction for alpha-fetoprotein (AFP), but the serum AFP level was normal. Additional distal gastrectomy with lymph node dissection revealed lymph node metastasis although there was no apparent finding of lymph node swelling by preoperative diagnostic imaging. This patient remains alive without disease for 3 years after surgery.
(Internal Medicine 43: 926–930, 2004)

Key words: Alpha-fetoprotein, early gastric carcinoma, immunohistochemistry, lymph node metastasis

Introduction

Alpha-fetoprotein (AFP)-producing gastric carcinoma (AFPGC) accounts for only 2–9% of gastric cancer (1). In AFPGC patients, liver metastases are often noted (2) and the tumor often has progressed to advanced gastric carcinoma at the time of diagnosis. In Japan, AFP-producing early gastric carcinoma (AFPEGC) is extremely rare and only 23 cases have been reported in the past 15 years (1–8). Herein we report the case of a man diagnosed with seronegative AFPEGC treated with endoscopic mucosal resection (EMR) and additional surgery.

For editorial comment, see p 889.

Case Report

A 67-year-old man visited our hospital for treatment of gastric cancer in September 2000. No specific family or past medical history was identified. Routine hematological examination and biochemical tests were within normal limits. In tumor marker examinations, carbohydrate antigen 19–9 was negative, however, carcinoembryonic antigen (CEA) was slightly high at 5.2 ng/ml (NR <5 ng/ml). Alpha-fetoprotein (AFP) was within normal limits at 3.4 ng/ml (NR <10 ng/ml).

Endoscopic examination of the upper digestive tract revealed a protruding lesion, about 25 mm in diameter, in the middle third of the greater curvature of the stomach (M area) (Fig. 1). The biopsy specimen obtained from the protruding lesion revealed moderately-differentiated adenocarcinoma. Examination by computed tomography (CT) revealed no abnormalities in the abdomen. There was no apparent finding of distant metastasis. Endoscopic ultrasonography (EUS) with a miniature probe of 20 MHz frequency using the water filling method revealed that the third layer of the gastric wall was uneven (Fig. 2). Thus we could not deny that the tumor had submucosal invasion. However, EUS was not sensitive enough to evaluate invasion to the submucosa in the present patient because this lesion was an elevated tumor and it was difficult to show the depth of the tumor by EUS.

The present patient was eager to undergo endoscopic resection. The patient underwent EMR for treatment under informed consent. EMR using an insulated-tip diathermic knife (IT-EMR) was performed as previously described (9). The protruding lesion was resected completely with a safe lateral and vertical margin, measuring 26×25 mm in size. Histo-

From Department of Internal Medicine, Shikoku Cancer Center, Matsuyama and *Department of Endoscopy, The Jikei University School of Medicine, Tokyo

Received for publication October 22, 2004; Accepted for publication June 2, 2004

Reprint requests should be addressed to Dr. Shoji Hirasaki, Shikoku Cancer Center, 13 Horino-uchi, Matsuyama 790-0007

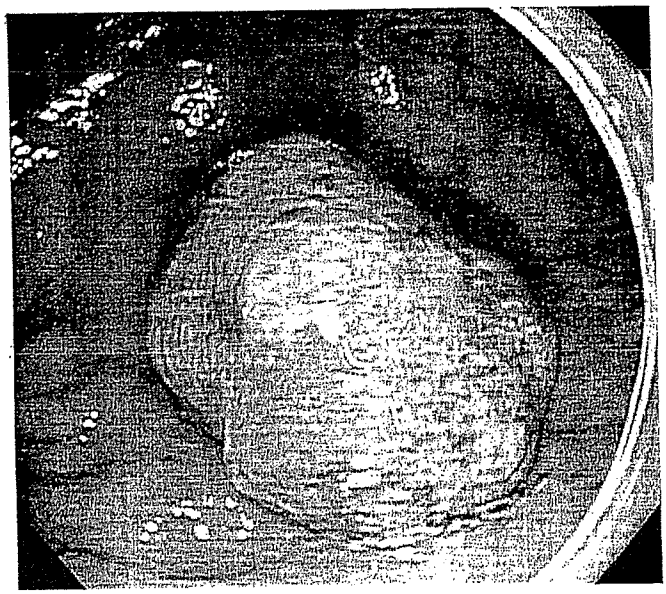

Figure 1. Endoscopic appearance of the gastric elevated lesion, about 25 mm in diameter, in the greater curvature of the middle third area (M area).

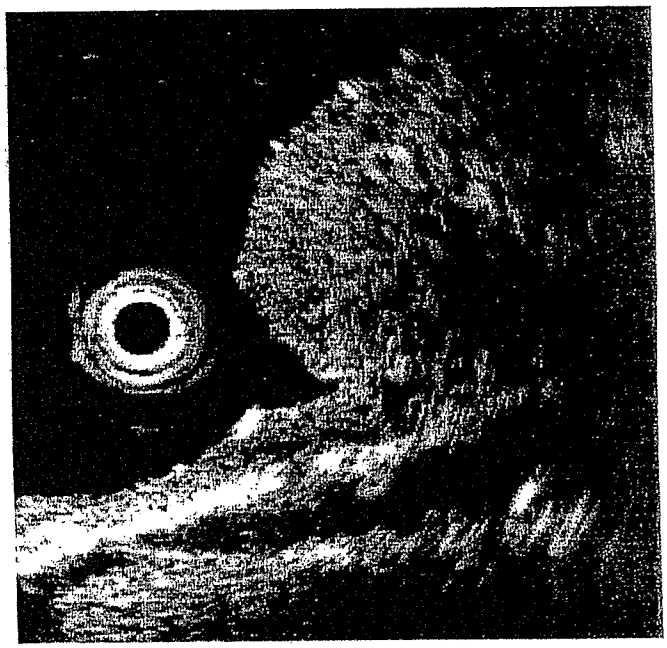

Figure 2. Endoscopic ultrasonography (EUS) shows that a hypoechoic protruding mass is mainly located in the first and second layers of the gastric wall and third layer is uneven.

logical examination of the protruding lesion revealed tumor invasion to the submucosal layer (Fig. 3A) with vessel invasion (Fig. 3B). The tumor was diagnosed as moderately-differentiated adenocarcinoma with submucosal invasion, ly2, and v1. The tumor revealed a solid papillary structure and included abnormal cells with clear cytoplasm and

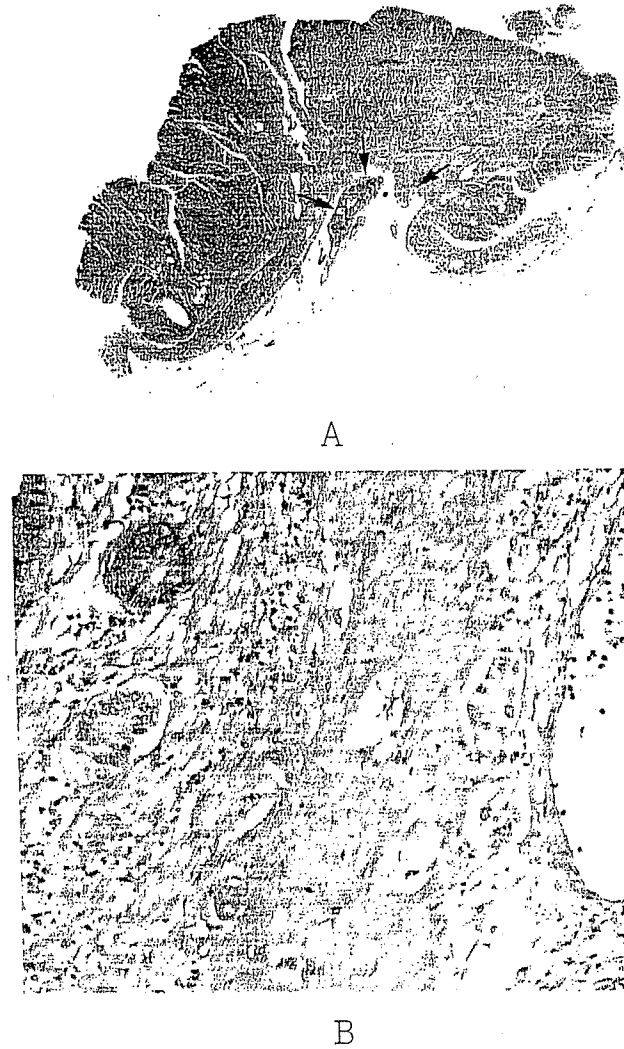

Figure 3. Microscopic findings of the resected tumor. (A) Low-power view showing a localized tumor expansion limited to the submucosal layer (black arrows) (HE stain, ×4). (B) Histological examination reveals apparent vessel invasion (HE stain, ×40).

hyperchromic round nuclei (Fig. 4). Thus, the tumor was suspected as AFPGC. Immunohistological studies were carried out for the resected specimens and the cancerous lesion showed a positive immunohistochemical reaction for AFP (Fig. 5). The malignant cells that invaded to the submucosal layer represented positive immunoreaction for AFP, however, tumor cells in the vessels were negative immunoreaction for AFP. AFP-producing tissue accounted for about 5% of this tumor.

Additional distal gastrectomy with radical lymph node dissection was carried out. Histological examination of the resected specimen revealed an ulcer after EMR and malignant tumor cells were removed. However, lymph node metastasis was seen at #3, 7, and 8a according to the Japanese classification of gastric carcinoma (10). The metastatic

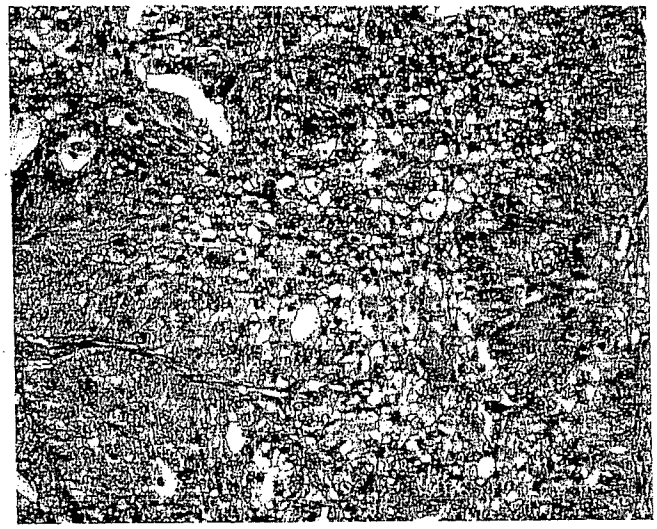

Figure 4. The tumor includes abnormal cells with clear cytoplasm and hyperchromic round nuclei (HE stain, ×20).

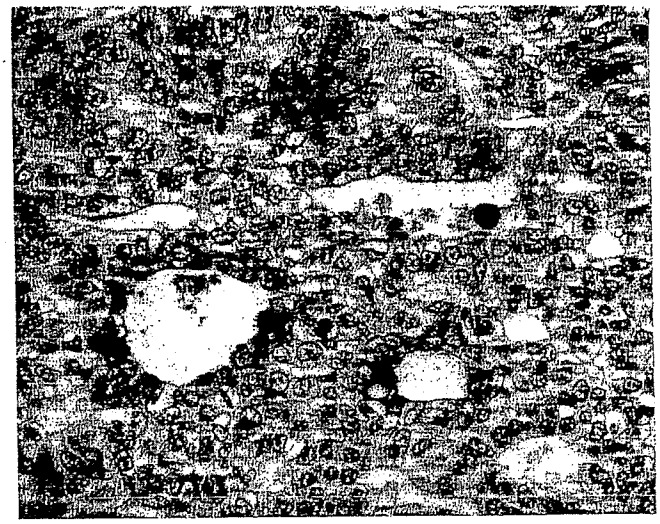

Figure 5. The tumor cells showing positive immunoreaction for alpha fetoprotein (×50).

Table 1. Summary of 24 Cases of Alpha Fetoprotein Producing

	Author	Age	Sex	Year	Serum AFP level at the onset	Sites	Symptoms	Size (cm)	Gross type
1	Ohta	73	F	1989	187	L	epigastric discomfort	2.8×1.5	IIa+IIc
2	Chang (2)	62	F	1990	146	M	ND	ND	IIa
3	Chang (2)	59	M	1990	4,800	L	ND	ND	IIc
4	Chang (2)	65	M	1990	<1	L	ND	ND	IIa
5	Kato	58	M	1990	91.3	L	none	2.7×1.7	IIa+IIc
6	Takiguchi	61	M	1991	125.1	L	none	2.0×2.0	IIa+IIc
7	Shirasaki	41	M	1992	23	U	epigastralgia	ND	IIa+IIc
8	Kubo	60	M	1992	44.9	L	none	2.5×2.5	IIa+IIc
9	Kubo	72	M	1992	21.2	L	epigastralgia	1.8×1.4	IIa+IIc
10	Kurita	61	M	1993	51.2	L	none	2.0×1.8	IIa+IIc
11	Umekawa (3)	61	M	1994	52		none	ND	IIc
12	Morikage (1)	59	F	1994	53.1	M	none	1.5×1.2	IIc+IIa
13	Suganuma (4)	61	M	1995	WNL	M	epigastralgia	ND	IIc
14	Hashimoto (5)	74	M	1996	WNL	M	appetite loss	2.3×2.0	IIc
15	Fujiya	79	F	1996	990	L	abdominal pain	ND	IIa+IIc
16	Kanazawa	66	M	1997	154.1	L	epigastralgia	ND	I
17	Tsurumachi	61	F	1997	910	L	epigastralgia	2.1×1.8	IIc
18	Taniguchi	65	M	1998	924	L	none	1	IIc
19	Higashi (6)	64	F	2001	170	L	epigastralgia	1.7×1.5	IIa+IIc
20	Shimoyama (7)	71	M	2001	113	U	none	2.3×2.0	IIc
21	Koyasaki	62	F	2002	287	M	appetite loss	5.0×2.5	IIc
22	Aoyagi (8)	73	M	2003	WNL	L	epigastralgia	4.0×4.0	I+IIa
23	Aoyagi (8)	76	M	2003	ND	L	none	2.5×2.5	IIa+IIc
24	Hirasaki	61	M	2003	3.4	M	none	2.6×2.5	I

ND: not described, M: male, F: female, L: lower one-third of stomach, M: middle one-third of stomach, U: upper one-third of stomach, wel: well differentiated adenocarcinoma, mod: moderately differentiated adenocarcinoma, por: poorly differentiated adenocarcinoma, mis: miscellaneous carcinoma, LN meta: Lymph node metastasis, Liver meta: Liver metastasis, sm: submucosal invasion, m: mucosal invasion,

tumor cells in these lymph nodes represented negative immunoreaction for AFP. The final diagnosis was T1, N2, M0 and stage II. Post operatively, the serum CEA level immediately decreased to the normal level (3.2 ng/ml). The postoperative course was uneventful and any additional chemotherapy was not performed according to informed consent because this patient was unwilling to receive adjuvant chemotherapy. He has been under close periodic observation, and the serum AFP and CEA levels have continuously been within normal limits during the follow-up period. There is no evidence of disease for 3 years after surgery.

Discussion

Generally, the prognosis for AFPGC is poor, because in such patients, liver metastases are often noted at the time of diagnosis (2). Although the tumor is an early gastric cancer, AFPGC tends to show liver metastasis, blood vessel invasion or lymphatic vessel invasion (4). In the present case, additional surgery was necessary after EMR because of the submucosal invasion and vessel invasion of the EMR resected specimen.

A review of the English and Japanese literature revealed 23 cases of AFPEGC reported in Japan in the past 15 years. Table 1 shows the 24 cases of AFPEGC reported in Japan in the past 15 years, including the present case (1–8). The male to female ratio was 17 : 7, the mean age was 64.4 years (range, 41 to 79 years), and the primary symptom at the time of onset was epigastralgia (29.2%). There were 10 asymptomatic patients (41.7%). AFPEGC sites were studied, and 15 of 24 patients (62.5%) had lesions in the lower third area (L area). As for gross type, IIa+IIc, IIc, IIa and I were most frequently observed in that order. IIa+IIc, IIc, IIa and I were macroscopically observed in 10, 8, 2, and 2 patients, respectively. Liver metastasis was seen in 2 patients (8.3%) and lymph node metastasis was seen in 18 (75.0%) of 24 patients at diagnosis, as seen in Table 1. Thus, lymph node metastasis should be generally taken into consideration for

Early Gastric Carcinoma Reported in Japan in the Past 15 Years

Depth	Histology	LN meta at the onset	Liver meta at the onset	Therapy	Adjuvant Chemotherapy	Chemotherapy after reccurence	Outcome
sm	mod	+	–	ope	–	no reccurence	alive
sm	por	+	–	ope	MMC	MMC+ADM (IAI)	dead
sm	por	+	–	ope	MMC+oral tegafur	CDDP+MMC+ ADM+5FU (IAI)	dead
sm	pap	+	–	ope	oral tegafur	–	dead
sm	por	–	+	ope	UFT(oral)+CDDP+ MMC+VP-16	no reccurence	alive
sm	mod～por	–	–	ope	–	no reccurence	alive
sm	pap	+	–	ope	–	ND	alive
sm	wel	+	–	ope	–	no reccurence	alive
sm	por	+	–	ope	–	+	dead
sm	por	–	–	ope	–	no reccurence	alive
sm	por	+	–	ope	–	ND	dead
sm	mod	–	–	ope	doxifluridine	no reccurence	alive
sm	por	+	–	ope	–	5FU+MMC (IAI)	dead
sm	wel～por	+	–	ope	UFT	5FU+CDDP+LV	alive
sm	mod	+	–	ope	–	no reccurence	alive
sm	por	+	–	ope	MMC carmofur+krestin	no reccurence	alive
sm	por	ND	+	ope	–	no reccurence	alive
m	wel	+	–	ope	5FU+CDDP 5FU+MMC+ADM (IAI)	no reccurence	alive
sm	mis	+	–	ope	–	no reccurence	alive
sm	mod	–	–	ope	5FU+CDDP+LV	no reccurence	alive
sm	por	+	–	ope	5FU+CDDP+LV	Epirubicin (IAI)	dead
sm	wel	+	–	ope	–	ND	dead
sm	por	+	–	ope	–	CDDP (IAI)	dead
sm	mod	+	–	EMR+ope	–	no reccurence	alive

ope: operation, m: mucosal invasion, EMR: endoscopic mucosal resection, UFT: uracil and tegafur, ADM: doxorubicin, VP-16: etoposide, 5FU: 5-fluorouracil, CDDP: cisplatin, LV: leucovorin, MMC: mitomycin C, IAI: intrahepatic arterial infusion.