opment of improved humanized mice will help to better evaluate the optimization of human immunotherapy.

Multifunctionality is the ability of T cells to exhibit multiple functions, including the simultaneous secretion of multiple cytokines, chemokines, or cytotoxic granules at the single-cell level.[51] The importance of T-cell multifunctionality has been reported in multiple animal infection models[52,53] and in humans infected with HIV, cytomegalovirus, hepatitis B virus, or tuberculosis.[53–60] We reported the importance of effector T-cell multifunctionality in antitumor immune response. Specifically, the appearance of multifunctional $CD8^+$ effector cytotoxic T cells *in vivo* is a critical determinant of effective immunological control of tumors. Regulatory T cells were found to play a role in the inhibition of transferred tumor antigen-specific T-cell multifunctionality.[33,39] In the present study, effector T-cell multifunctionality appeared to correlate with the quality of T-cell responses in adoptive T-cell therapy utilizing genetically-engineered human lymphocytes (Figs 6,7). The peptide vaccination did not significantly change the percentage of human $CD3^+CD8^+$ cells in the PBMC of NOG mice (data not shown). The TCR-transduction efficiency in this study was not very high in general. We found that the combination of vaccination with the adoptive transfer of antigen-specific T cells increased effector T-cell multifunctionality and made the antitumor effect visible, even with a low number of specific TCR-transduced T cells transferred. The unmodified cells with background reactivity were the IFN-γ single producers. We speculate that these cells are positive for IFN-γ because of their non-specific activation due to GVH reaction.

To our knowledge, this study represents the first demonstration *in vivo* of an antitumor effect following the adoptive transfer of human lymphocytes genetically engineered to express a TCR specific for MAGE family antigen. The retroviral vector used in this report is currently under evaluation in a phase I clinical trial designed to treat patients with MAGE-A4-expressing esophageal cancer.

In summary, our data suggest that adoptive cell therapy with human lymphocytes engineered to express MAGE-A4-specific TCR through retroviral transduction is a promising strategy to treat patients with MAGE-A4-expressing tumors. Combination therapy with gene-modified cell-adoptive transfer and *in vivo* vaccination might improve antitumor efficacy, even with low numbers of transferred tumor-reactive T cells. These data support the rationale to explore clinical trials utilizing gene-modified lymphocytes prepared using the vector described in this report.

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Disclosure Statement

No potential conflicts of interest were disclosed.

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Original Article

Effects of Ghrelin Administration During Chemotherapy With Advanced Esophageal Cancer Patients

A Prospective, Randomized, Placebo-Controlled Phase 2 Study

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BACKGROUND: Cisplatin reduces plasma ghrelin levels through the 5-hydroxytryptamine (5-HT) receptor. This may cause cisplatin-induced gastrointestinal disorders and hinders the continuation of chemotherapy. The authors of this report conducted a prospective, randomized phase 2 trial to evaluate the effects of exogenous ghrelin during cisplatin-based chemotherapy. **METHODS:** Forty-two patients with esophageal cancer who were receiving cisplatin-based neoadjuvant chemotherapy were assigned to either a ghrelin group (n = 21) or a placebo group (n = 21). They received either intravenous infusions of synthetic human ghrelin (3 μg/kg) or saline twice daily for 1 week with cisplatin administration. The primary endpoint was changes in oral calorie intake, and the secondary endpoints were chemotherapy-related adverse events; appetite visual analog scale (VAS) scores; changes in gastrointestinal hormones and nutritional status, including rapid turnover proteins, and quality of life (QoL) estimated with the European Organization for Research and Treatment of Cancer QoL core questionnaire (QLQ-C30). **RESULTS:** Two patients were excluded from the final analysis: One patient suspended ghrelin administration because of excessive diaphoresis, and another patient in the placebo group failed to monitor the self-questionnaire. Food intake and appetite VAS scores were significantly higher in the ghrelin group than in the placebo group (18.2 ± 5.2 kcal/kg/day vs 12.7 ± 3.4 kcal/kg/day [$P = .001$] and 6.2 ± 0.9 vs 4.1 ± 0.9 [$P < .0001$], respectively). Patients in the ghrelin group had fewer adverse events during chemotherapy related to anorexia and nausea than patients in the control group. Significant deterioration was noted after chemotherapy in the placebo group in QoL scores, appetite, nausea and vomiting, and global health status. **CONCLUSIONS:** Short-term administration of exogenous ghrelin at the start of cisplatin-based chemotherapy stimulated food intake and minimized adverse events. ***Cancer* 2012;118:4785-94.** © *2012 American Cancer Society.*

KEYWORDS: ghrelin, esophageal cancer, food intake, appetite, cisplatin-based chemotherapy.

INTRODUCTION

Neoadjuvant and/or adjuvant chemotherapy using multiple antitumor agents is an important component of any therapeutic regimen for advance-stage solid tumors.[1] Cisplatin plays a central role in the success of such multidrug chemotherapy regimens for various cancers[2]; however, it is also associated with an assortment of adverse effects, including nephrotoxicity, myelosuppression, and gastrointestinal disorders like nausea, vomiting, and appetite loss. These gastrointestinal symptoms generally are nonlethal and reversible; however, their high frequency and strength can strongly impair the patient's quality of life (QoL) and, in general, may preclude the completion of chemotherapy.

The acute phase of cisplatin-induced gastrointestinal disorders involve increased serotonin (5-hydroxytryptamine [5-HT]) secretion from enterochromaffin cells.[3] Consequently, a 5-HT3-receptor antagonist was developed and is widely used for patients with cancer who are receiving cisplatin-based chemotherapy.[4] Despite this advance, many patients still suffer from gastrointestinal disorders because of cisplatin, especially in the later phases of treatment.

Ghrelin is an endogenous ligand for the growth hormone (GH) secretagogue receptor and is secreted predominantly by gastric endocrine cells.[5] It induces dose-dependent, GH-releasing activity[5,6]; stimulates appetite and food intake; and triggers a positive energy balance through a central mechanism involving hypothalamic neuropeptides.[5-9] In rodents, ghrelin increases GH secretion, feeding, and body weight when administered centrally or peripherally.[7-9] We also reported

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previously that intravenous administration of ghrelin enhanced oral feeding and diminished weight loss in patients who underwent total gastrectomy[10] and esophagectomy.[11]

In rodents, cisplatin markedly decreased plasma ghrelin concentrations, whereas the administration of exogenous ghrelin improved cisplatin-induced decreases in food intake.[12,13] These observations suggested that ghrelin also may be effective in minimizing the gastrointestinal disorders induced by cisplatin in humans, although there are huge differences in feeding activity between the 2 species. Accordingly, we undertook a randomized clinical trial to elucidate the effect of exogenous ghrelin on patients with esophageal cancer who were receiving cisplatin-based neoadjuvant chemotherapy.

MATERIALS AND METHODS

Patients

This prospective, randomized, placebo-controlled phase 2 study enrolled 42 patients with advanced esophageal cancer who received cisplatin-based neoadjuvant chemotherapy. The Human Ethics Review Committee of Osaka University School of Medicine approved the study protocol, and a signed consent form was obtained from each enrolled patient before study entry in accordance with the Declaration of Helsinki. This study was registered on the University Hospital Medical Information Network (R000005924). It began in February 2010, and enrollment of patients ended in January 2011. The eligibility criteria for the study were as follows: 1) histopathologically confirmed squamous cell carcinoma of the esophagus; 2) stage II or III disease according to criteria of the International Union Against Cancer (UICC), sixth edition[14]; 3) ages 20 to 80 years; 4) no esophageal obstruction by tumor and capacity for oral intake of soft solid foods; 5) adequate function of major organs; 6) no other active malignancy; 7) an Eastern Cooperative Oncology Group performance status (PS) of 0 or 1; and, 8) provision of written informed consent. The exclusion criteria for the study were as follows: 1) pregnant or potentially (willingly) pregnant women; 2) a past history of other chemotherapy or radiotherapy; and, 3) patients judged to be ineligible by the investigator.

A coordinating center (a section of the Department of Gastroenterological Surgery, Osaka University Medical School) was responsible for creating the treatment allocation code using a computer-generated randomization table with a statistician. Patients were randomized at a 1:1 ratio to receive intravenous infusion of either synthetic human ghrelin (3 μg/kg) or placebo (saline). Treatment allocation was arranged before the beginning of chemotherapy. The study was performed in a single-blind manner, ie, without knowledge of allocation to the patients.

Calculation of Sample Size

We estimated that oral intake of food calories during the study period in the placebo group would be 1600 ± 300 kcal/day. The power calculation was based on a 20% improvement by ghrelin administration in oral food intake calories, with a power of 85% and an α value of 5%, requiring at least 17 patients per study group. Assuming that approximately 20% of patients in each group would not complete the study, the initial proposal aimed to recruit 20 patients in each group.

Neoadjuvant Chemotherapy Regimen

The enrolled patients received cisplatin-based chemotherapy. This was a regimen consisting of either 5-fluorouracil, cisplatin, and doxorubicin (ACF)[3,15] or 5-fluorouracil, cisplatin, and docetaxel (DCF).[16] Both regimens entailed 2 treatments every 4 weeks. Specifically, the ACF regimen comprised cisplatin (70 mg) and doxorubicin (35 mg) on day 1 and a continuous infusion of 5-fluorouracil (700 mg/day) for 7 days, whereas the DCF regimen comprised cisplatin (70 mg) and docetaxel (70 mg) on day 1 and then a continuous infusion of 5-fluorouracil (700 mg/day) for 5 days. Supportive therapy and prophylaxis against expected side effects was provided. All patients were premedicated with intravenous ramosetron hydrochloride (0.3 mg), a representative 5-HT3 receptor antagonist. This was infused 1 hour before the administration of cisplatin on day 1 and every morning thereafter on days 2 through 7 (ACF regimen) or days 2 through 5 (DCF regimen). Hypersensitivity reactions were treated prophylactically with intravenous dexamethasone (8 mg), which was infused 1 hour before the administration of cisplatin. Adequate hydration was ensured before and after cisplatin infusion. Additional antiemetics or steroid preparations were recommended in case of grade 3 or greater anorexia, nausea, and vomiting according to toxicity grading criteria from the Common Terminology Criteria for Adverse Events version 4.0 (CTCAE).[17] After completion of the second cycle of neoadjuvant chemotherapy, the patient underwent curative resection, ie, subtotal esophagectomy with reconstruction by gastric tube, together with 2-field or 3-field lymphadenectomy.[18]

Evaluation of Adverse Events and Criteria for Dose Modifications

Adverse events were evaluated each day of chemotherapy and were scored by the most severe event in the first cycle (days 1-28) based on the toxicity grading criteria from the

CTCAE by each primary physician. Before starting the second cycle of chemotherapy, patients were required to have grade <2 hematologic toxicity. When patients did not recover within a 2-week delay or had grade 4 nonhematologic toxicity in the first cycle, the chemotherapy was discontinued, and surgical resection was considered.

Dose modifications in the second cycle were based on treatment-related adverse events recorded in the first cycle. In the ACF regimen, the doses of cisplatin and doxorubicin were reduced by 20% for grade 4 neutropenia that lasted >5 days, febrile neutropenia grade ≥3, and thrombocytopenia grade ≥3. In the DCF regimen, the doses of cisplatin and docetaxel were reduced by 20% for the same hematogenic toxicity. The dose of cisplatin was reduced by 20% in the second cycle in both regimens after a rise in serum creatinine level above 1.5 mg/dL during the first cycle. The dose of 5-fluorouracil was reduced by 20% for grade ≥3 diarrhea and mucositis. After completing 2 cycles of neoadjuvant chemotherapy, all patients were restaged by endoscopy and computed tomography to evaluate the clinical response to chemotherapy 2 weeks after the completion of chemotherapy. Clinical responses were categorized according to criteria based on the World Health Organization response criteria for measurable disease and the Japanese Society for Esophageal Diseases.[19]

Study Protocol

The study protocol is summarized in Figure 1A. Patients who were assigned to the ghrelin group received ghrelin treatment at a dose of 3 μg/kg body weight diluted in 50 mL saline given over 30 minutes twice daily (before breakfast and before dinner) for 7 consecutive days (days 1-7), as in our previous studies.[10,11] Synthetic ghrelin was prepared and supplied as described previously.[10,11] Patients in the placebo group received a corresponding placebo (pure saline) infusion in the same fashion. All participants received the same protocol of intravenous infusion in both groups, ie, 3000 mL/day from days 1 to 3 and 2000 mL/day from days 4 to 7 of chemotherapy, including 43 g glucose, 35 millequivalents (mEq) sodium, 20 mEq potassium, 35 mEq chloride, and 20 mEq lactate in 1000 mL.

Endpoints

The primary endpoint of this study was alteration in oral calorie intake from day 1 to day 7 of chemotherapy. Patients in this study were served standard meals and were allowed to receive extra food if desired. All dietary intake calories were calculated by a national registered dietitian at Osaka University Hospital by measuring the weight of each dish diet before and after every meal.[10,11] The secondary endpoints included changes in appetite, adverse events, QoL, body weight, nutritional status, hormonal assays, and blood tests. Appetite profiles were measured using a 100-mm visual analog scale (VAS), with the questions "How hungry are you?" and "How full do you feel?," which were anchored with "0 not at all" and "100-extremely." Patients were instructed to rate themselves by selecting the scale before each meal that was most appropriate to their feeling at that time. The mean VAS score was calculated each day. Questionnaires included the European Organization for Research and Treatment of Cancer core QoL questionnaire (QLQ-C30) before and after chemotherapy (day 8).[20] The QLQ-C30 contains 5 functional scales (physical, role, cognitive, emotional, and social), 3 symptom scales (fatigue, pain, and nausea/vomiting), a global health/QoL scale, and 6 single items (dyspnea, insomnia, appetite loss, constipation, diarrhea, and financial difficulties). All scale scores and single items scores range from 0 to 100. A high score for a functional scale represents a higher ("better") level of functioning, whereas a high score for a symptom scale or item represents a higher ("worse") level of symptoms.

Blood samples were collected before breakfast after an overnight fast before chemotherapy and on Days 3 and 8 of chemotherapy. The samples were transferred immediately into chilled tubes containing disodium ethylenediamine tetra-acetic acid and aprotinin, centrifuged at 4°C, separated for serum sampling, and stored at −50°C. The plasma samples were mixed with a 10% volume of 1 M hydrochloric acid before storing at −50°C. Plasma acyl-ghrelin and desacyl-ghrelin concentrations were measured with a sandwich-type enzyme immunoassay kit according to the protocol supplied by the manufacturer (Mitsubishi Kagaku Iatron, Inc., Tokyo, Japan).[21] Total plasma ghrelin concentration was calculated as acyl-ghrelin plus desacyl-ghrelin concentration. Serum GH, insulin, and leptin concentrations were measured using a GH "Daiichi" kit (TFB, Inc., Tokyo, Japan), a chemiluminescent enzyme immunoassay (Fujirebio, Inc., Tokyo, Japan), and a human leptin radioimmunoassay (RIA) kit (Linco Research Inc., St. Charles, Mo), respectively. Serum insulin-like growth factor-1 (IGF-1) levels were measured by RIA (SRL Company Ltd., Tokyo, Japan).

Statistical Analysis

Continuous variables are expressed as the mean ± standard deviation unless stated otherwise. Statistical differences between groups were calculated by using the Student *t* test, the Fisher exact test, the Mann-Whitney test, or the chi-square test, as appropriate. Comparisons of the time

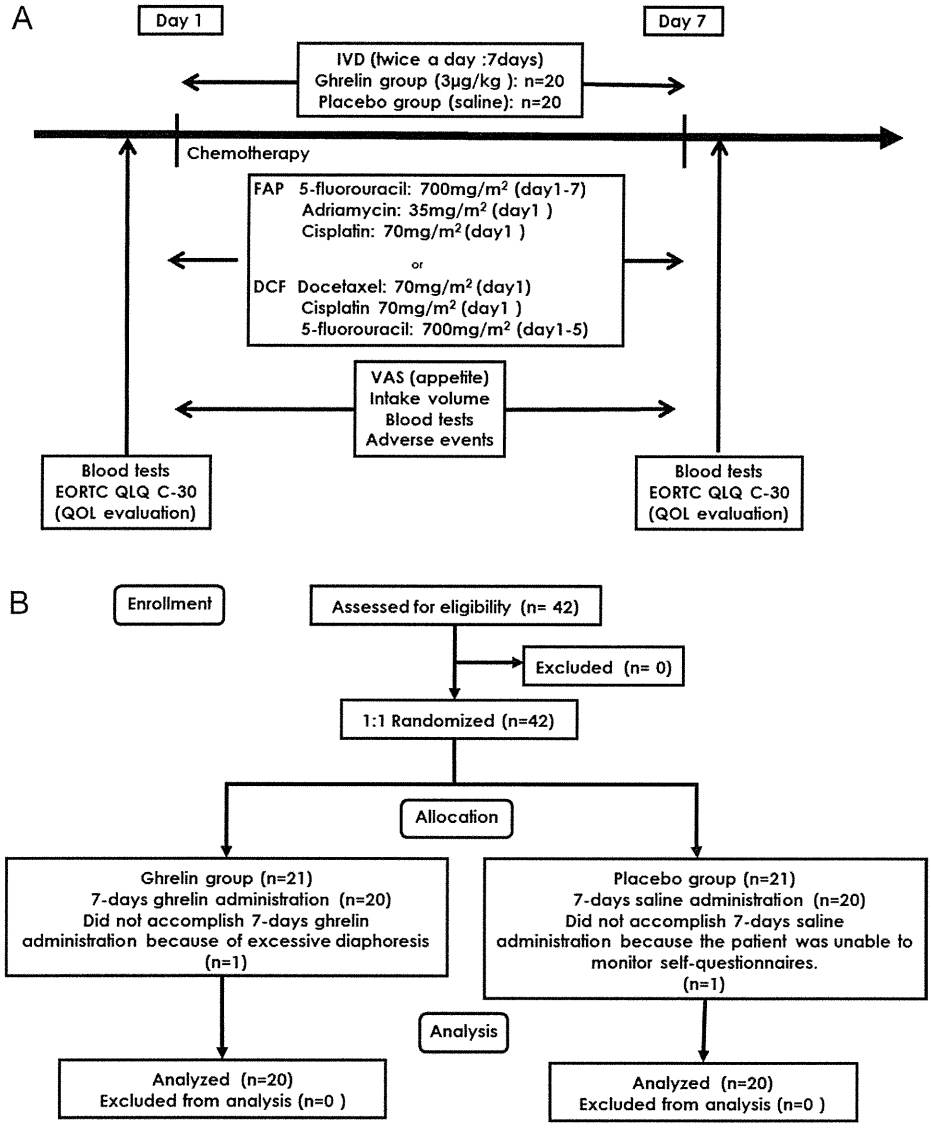

Figure 1. (A) This is a flow diagram of process through the trial. (B) The study protocol is illustrated. IVD indicates intravenous drip; FAP, combined 5-fluorouracil, doxorubicin (Adriamycin), and cisplatin; DCF, combined docetaxel, cisplatin, and 5-fluororuacil; VAS; visual analog scale; EORTC QLQ C-30, European Organization for Research and Treatment of Cancer Core-30 Quality-of-Life Questionnaire; QOL, quality of life.

course of food intake calories and appetite score were tested by using a 2-way repeated-measures analysis of variance (ANOVA). Statistical significance was set at $P < .05$. All calculations were performed using the JMP (version 9.0) software program (SAS Institute Inc, Cary, NC).

RESULTS

Patient Characteristics

In total, 42 enrolled patients were randomized into either the ghrelin group (21 patients) or the placebo group (21 patients). One patient (4.8%) in the ghrelin group who developed excessive diaphoresis during ghrelin infusion, equivalent to grade 2 according to CTCAE, and another patient (4.8%) in the placebo group who was unable to monitor the self-questionnaire because of general fatigue were excluded from the analysis (Fig. 1B). Table 1 lists the demographic and clinical characteristics of all patients. There were no significant differences in the background characteristics, including age, sex, body mass index, localization of cancer, clinical cancer staging, or chemotherapy regimen.

Table 1. Patient Characteristics

Parameter	No. of Patients: Ghrelin Group	Placebo Group	*P*
No. of patients	20	20	
Age: Mean±SD, y	65.8±5.2	61.8±10.9	.14
Sex			.28
Men	19	17	
Women	1	3	
BMI: Mean±SD, kg/m^2	21.6±.3	21.0±2.7	.44
Tumor localization			.27
Upper thoracic	4	1	
Middle thoracic	9	9	
Lower thoracic	7	10	
Clinical UICC TNM stage			
Tumor classification			.45
T1	0	0	
T2	6	4	
T3	8	12	
T4	6	4	
Lymph node status			.51
N0	8	6	
N1	12	14	
Metastasis classification			.43
M0	17	15	
M1	3	5	
Disease stage			.38
I	0	0	
II	9	7	
III	8	8	
IV	3	5	
Chemotherapy regimen			.74
ACF	13	12	
DCF	7	8	

Abbreviations: ACF: doxorubicin, cisplatin, and 5-fluorouracil; BMI, body mass index; DCF: docetaxel, cisplatin and 5-fluorouracil; SD, standard deviation; UICC, International Union Against Cancer.

Effect of Ghrelin on Dietary Intake and Appetite Scoring

The mean dietary intake gradually decreased after cisplatin administration to reach the lowest level on days 5 through 7. After completing chemotherapy, it took another 4 to 7 days for oral intake to recover and to allow hospital discharge. Although patients in the ghrelin and placebo groups reflected this trend, the decline in dietary intake with chemotherapy was significantly less in the ghrelin group compared with the placebo group (18.1 kcal/kg/day vs 12.7 kcal/kg/day overall), especially at day 1 (26.7 kcal/kg/day vs 23.1 kcal/kg/day) compared with day 7 (15.0 kcal/kg/day vs 8.5 kcal/kg/day) (Fig. 2A). In other words, the improved oral food intake because of ghrelin administration was more significant in the later phase of chemotherapy (repeated-measures ANOVA: ghrelin group vs placebo group, $P = .0027$). Changes in the VAS score reflected the changes in dietary intake between the 2 groups with a significant difference among them (repeated-measures ANOVA: ghrelin group vs placebo group, $P < .0001$, Fig. 2B). Notably, the appetite scores recovered more quickly after day 4 of chemotherapy in the ghrelin group than in the placebo group.

Effect of Ghrelin on Nutritional and Hormone Status

Table 2 details the blood test results before and after chemotherapy (day 8) in the ghrelin and placebo groups. There were no significant differences in nutritional parameters before chemotherapy, including hemoglobin, albumin, lymphocyte numbers, cholinesterase, total cholesterol, and the rapid turnover proteins (RTP) (prealbumin, retinol-binding protein, and transferrin). In the placebo group, significant declines after chemotherapy were observed for hemoglobin, prealbumin, and transferrin, but not for the other nutritional parameters tested. This RTP finding is consistent with ghrelin preventing nutritional deterioration because of chemotherapy compared with the placebo group (prealbumin: 26.4 ± 4.6 mg/dL vs 21.7 ± 2.8 mg/dL [$P = .042$]; transferrin: 205 ± 18 mg/dL vs 162 ± 32 mg/dL [$P = .037$]).

With respect to ghrelin and associated hormones, plasma total ghrelin levels (acyl-ghrelin plus desacyl-ghrelin) significantly decreased after chemotherapy, accounting for 61% of the baseline values (before chemotherapy) in the placebo group. GH, a target hormone for ghrelin, and IGF-1, a mediator of GH, consistently tended to decrease after chemotherapy. However, despite the poor dietary intake during chemotherapy, leptin tended to decrease rather than increase after chemotherapy. There were no significant differences in plasma ghrelin levels between the groups before and after chemotherapy because of its rapid turnover. Likewise, the levels of GH, IGF-1, insulin, and leptin did not differ between the ghrelin and placebo groups.

Adverse Events

Table 3 lists the hematologic and nonhematologic adverse events during the first cycle of chemotherapy. Diaphoresis is a known physiologic effect of ghrelin. One patient with grade 2 diaphoresis was excluded, whereas another with grade 1 diaphoresis completed the study protocol and was included in the analysis. Anorexia and nausea are the most common toxicities reported with cisplatin-based chemotherapy. In our study, grade ≥3 symptoms were noted in 55% (anorexia) and 60% (nausea) of patients in the placebo group. Ghrelin administration significantly reduced

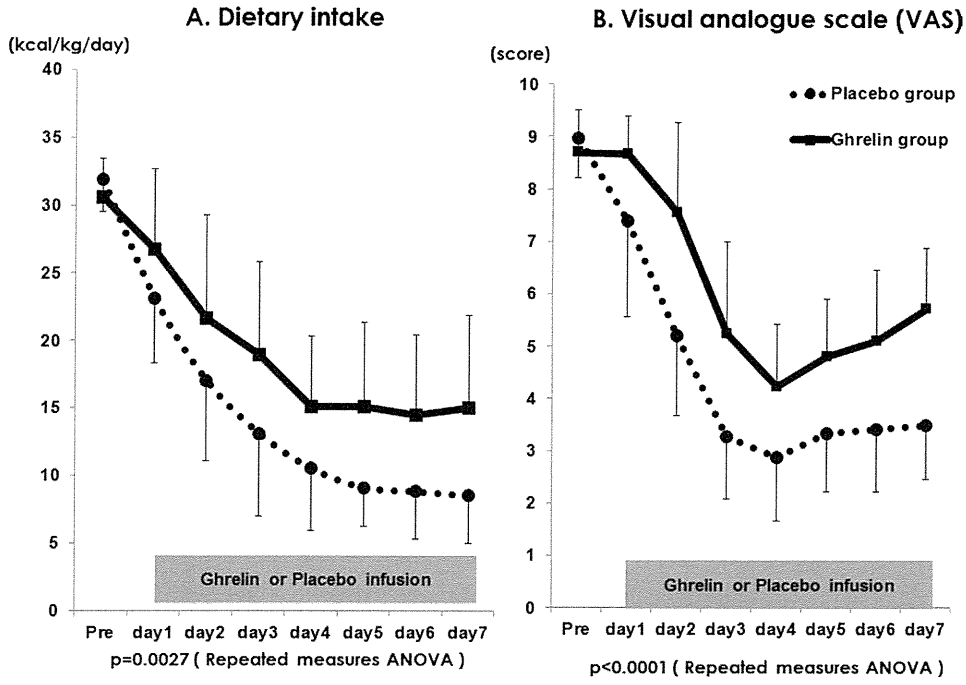

Figure 2. (A) Serial changes in dietary calorie intake are illustrated before and during chemotherapy in the ghrelin group (solid squares) and the placebo group (solid circles). (B) The visual analog scale score for appetite was similar in the 2 groups before chemotherapy. Data shown are means ± standard deviations. ANOVA indicate analysis of variance.

these adverse effects to 15% and 20%, respectively (anorexia: ghrelin group vs placebo group, $P = .016$; nausea: ghrelin group vs placebo group, $P = .012$). Other adverse effects, including myelosuppression, renal toxicity, and stomatitis, did not differ significantly between the 2 groups.

Treatment Outcome

Dose modifications were necessary in the second cycle of chemotherapy for 6 patients (30%) in the ghrelin group and for 10 patients (50%) in the placebo group according to the criteria for dose modifications. Thus, patients in the ghrelin group displayed less toxicity from chemotherapy than those in the placebo group during the second cycle, although the difference did not reach statistical significance ($P = .17$). Ghrelin administration tended to reduce the length of hospital stay in the ghrelin group compared with the placebo group (18.4 days vs 23.5 days; $P = .12$). The objective tumor response rate after the second cycle of chemotherapy was not different between the 2 groups: In the ghrelin group, 13 patients achieved a partial response, 6 patients had no change, and 1 patient had progressive disease (PD); whereas, in the placebo group, 13 patients had a partial response, 4 patients had no change, and 3 patients had progressive disease. After 2 cycles of chemotherapy, 16 patients in the ghrelin group and 15 patients in the placebo group underwent curative resection. There were no significant differences in major surgical complications between the 2 groups.

Quality-of-Life Evaluation

Patients in the ghrelin group reported significantly better overall global health status scores after chemotherapy than patients in the placebo group (52 ± 18 vs 26 ± 13, respectively; $P < .0001$), although there were no significant differences in the functional scale parameters. With respect to the symptom scale scores and items, patients in the ghrelin group scored better after chemotherapy than patients in the placebo group on nausea/vomiting (ghrelin group vs placebo group: 16 ± 14 vs 36 ± 29; $P < .0001$) and appetite loss (26 ± 14 vs 54 ± 22; $P < .0001$). Although the differences were not statistically significant, patients in the ghrelin group scored better after chemotherapy than patients in the placebo group on fatigue ($P = .082$). There were no significant differences in other symptom scales or items (Table 4).

DISCUSSION

In this prospective, randomized trial, we demonstrated that the administration of synthetic ghrelin during cisplatin-based neoadjuvant chemotherapy successfully increased food intake and appetite and decreased the adverse effects of chemotherapy. To our knowledge, this

Table 2. Results of Laboratory Tests, Nutritional Status, and Hormone Assays

Variable[a]	Mean±SD Value: Ghrelin Group	Placebo Group	*P*
Hemoglobin, g/dL			
Before	11.2±0.8	11.5±1.3	.35
After	10.4±0.7	10.2±1.1[a]	.41
Albumin, g/dL			
Before	3.6±0.3	3.4±0.4	.78
After	3.2±0.5	3.3±0.6	.67
Lymphocytes, /μL			
Before	1590±350	1620±400	.56
After	1450±320	1540±350	.58
Cholinesterase, IU/L			
Before	225±65	212±48	.21
After	205±45	190±38	.25
Total cholesterol, mg/dL			
Before	138±45	148±42	.46
After	144±42	142±48	.36
Rapid turnover protein			
Prealbumin, mg/dL			
Before	24.6±6.6	26.2±5.8	.65
After	26.4±4.6	21.7±2.8[a]	.042
Retinol binding protein, mg/dL			
Before	3.5±0.8	3.8±0.6	.31
After	3.8±0.8	3.6±0.9	.37
Transferrin, mg/dL			
Before	210±38	235±23	.45
After	205±18	162±32[a]	.037
Hormones			
Total ghrelin, fmol/mL			
Before	144±65	135±58	.34
After	94±48[a]	82±32[a]	.42
Growth hormone, ng/mL			
Before	1.8±1.5	1.7±0.8	.81
After	1.5±0.9	1.4±0.8	.26
Insulin-like growth factor-1, ng/mL			
Before	144±52	152±45	.58
After	134±47	141±42	.47
Insulin, μIU/mL			
Before	6.4±3.2	8.2±4.1	.54
After	5.3±2.4	6.3±3.8	.42
Leptin, ng/mL			
Before	3.2±1.8	2.9±1.7	.76
After	2.1±0.4	2.5±0.5	.32

Abbreviations: SD, standard deviation.
[a] $P < .05$ for before versus after.[b]Before indicates before chemotherapy; After: after chemotherapy (day 8).

Table 3. Adverse Events Encountered During Chemotherapy

Adverse Events[a]	No. of Events: Ghrelin Group	Placebo Group	*P*
Neutropenia			.49
Grade 0	4	4	
Grade 1-2	9	6	
Grade 3-4	7	10	
Lymphopenia			.75
Grade 0	12	11	
Grade 1-2	8	9	
Grade 3-4	0	0	
Anemia			.75
Grade 0	14	13	
Grade 1-2	5	6	
Grade 3-4	1	1	
Thrombocytopenia			.59
Grade 0	16	17	
Grade 1-2	2	3	
Grade 3-4	2	0	
Renal toxicity			.91
Grade 0	13	13	
Grade 1-2	7	6	
Grade 3-4	0	1	
Diaphoresis			.32
Grade 0	19	20	
Grade 1-2	1	0	
Grade 3-4	0	0	
Anorexia			.016
Grade 0	4	2	
Grade 1-2	13	7	
Grade 3-4	3	11	
Nausea			.012
Grade 0	3	1	
Grade 1-2	13	7	
Grade 3-4	4	12	
Vomiting			.35
Grade 0	5	4	
Grade 1-2	12	10	
Grade 3-4	3	6	
Diarrhea			.77
Grade 0	9	10	
Grade 1-2	10	9	
Grade 3-4	1	1	
Stomatitis			.77
Grade 0	3	3	
Grade 1-2	15	14	
Grade 3-4	2	3	

[a] Adverse events were evaluated according to toxicity grading criteria from version 4.0 of the *Common Terminology Criteria for Adverse Events*.

is the first report on the usefulness of ghrelin administration during cisplatin-based chemotherapy in humans.

It has been reported that acute gastrointestinal disorders caused by cisplatin involve 5-HT secretion from the enterochromaffin cells in association with 5-HT3 receptors.[3,4] Therefore, the administration of a 5-HT3 receptor antagonist is effective in the suppression of cisplatin-induced nausea and vomiting that occur within 24 hours after administration.[4] However, late-phase chemotherapy-induced anorexia, nausea, and vomiting still are difficult to adequately control. In the current study, the mean

Table 4. Quality-of-Life Scores

QLQ-C30[a]	Mean±SD Score: Ghrelin Group	Placebo Group	*P*
Global health status score			
Before	78±30	74±22	.51
After	52±18	26±13	< .0001
Functional scales			
Physical functioning			
Before	86±8	92±10	.62
After	78±20	72±18	.42
Role functioning			
Before	80±12	88±8	.43
After	68±16	70±15	.29
Emotional functioning			
Before	78±14	82±12	.26
After	70±18	68±14	.44
Cognitive functioning			
Before	88±11	90±10	.72
After	86±14	88±18	.67
Social functioning			
Before	84±20	82±22	.54
After	82±16	78±14	.52
Symptom scales/items			
Fatigue			
Before	12±6	14±8	.37
After	22±11	34±16	.082
Nausea/vomiting			
Before	5±6	4±7	.62
After	16±14	36±29	< .0001
Pain			
Before	8±6	7±9	.47
After	10±11	12±14	.59
Dyspnea			
Before	8±14	7±13	.68
After	8±12	7±14	.66
Insomnia			
Before	12±8	14±12	.75
After	20±12	19±14	.37
Loss of appetite			
Before	8±14	7±13	.43
After	26±14	54±22	< .0001
Constipation			
Before	7±13	8±12	.29
After	12±18	14±20	.21
Diarrhea			
Before	12±14	12±18	.69
After	22±18	26±22	.32
Financial difficulties			
Before	16±22	18±17	.58
After	18±24	16±21	.72

Abbreviations: SD, standard deviation.
[a] Before indicates before chemotherapy; After: after chemotherapy (day 8).

oral intake of calories decreased significantly to about 25% of the baseline level at day 8 after chemotherapy despite the use of a 5-HT3 antagonist.

Several observations suggest that ghrelin may play an important role in the delayed cisplatin-induced gastrointestinal effects. In rodents, a single cisplatin administration caused a transient decrease in plasma ghrelin concentration and prolonged suppression of both food intake and body weight loss.[22] Cotreatment with a 5-HT3 antagonist did not result in the recovery of ghrelin levels or dietary activity in that experiment. In our clinical study, we observed that chemotherapy that included cisplatin reduced plasma ghrelin levels to 67% and 57% of the baseline levels on days 3 and 8, respectively. In addition, there was a close relation between the extent of decline in plasma ghrelin, nutritional status, and adverse events of chemotherapy.[23] In the current trial, we demonstrated that the administration of synthetic ghrelin during chemotherapy successfully increased food intake and appetite. This effect may be explained by the effect on the GH/IGF-1 axis. The growth-promoting effect of GH is mediated, at least in part, by IGF-1.[24] However, serum GH and IGF-1 levels were stable in both groups, probably because of the rapid turnover of GH. Although this phenomenon was reported previous in earlier studies,[14,24] we should have measured GH and IGF-1 in a brief period.

5-HT3 antagonist also was administered in the current clinical study. Taken together, the acute and delayed effects of cisplatin on gastrointestinal functions may involve different mechanisms, and the delayed effects, which seemingly are not mediated through the 5-HT3 receptor, affect nutrition status in cancer patients more strongly than the acute effects.

Conversely, recent reports indicate that both the 5-HT2C receptor and the 5-HT2B receptor, but not the 5-HT3 receptor, mediate cisplatin-induced ghrelin suppression in rodents.[13,22] The 5-HT2B receptor is distributed mainly in gastrointestinal smooth muscle,[25] and the 5-HT2C receptor is localized in the central nerve system.[26] Vagal nerve function may regulate afferent and efferent signaling, which controls ghrelin secretion through these 5-HT2B and 5-HT2C receptors. However, in our previous study, ghrelin was administered to patients who had undergone gastrectomy and esophagectomy, which also included truncal vagotomy, and we observed significant effects on appetite and body weight increase.[10,11] Therefore, the association between ghrelin signaling and the vagal nerve remains unresolved.[27] In the literature, urinary 5-hydroxyindole acetic acid (5-HIAA), the major metabolite of 5-HT, increased rapidly and subsequently returned to baseline within the first 24 hours after cisplatin administration, and it was associated strongly with chemotherapy-induced emesis.[3,4,12,28] In the current study, serum 5-HT and 5-HIAA levels on days 3 and 8 of chemotherapy did not increase significantly compared

with baseline values (data not shown). Thus, because plasma ghrelin undergoes rapid turnover, our observation regarding 5-HIAA suggests that 5-HT does not directly control ghrelin secretion.

In other studies, substance P and neurokinin-1 (NK-1) receptor contributed to the delayed emetic symptoms associated with chemotherapy.[29] Accordingly, an NK-1 receptor antagonist could inhibit the binding of substance P to the NK1 receptor in the vomiting center.[29] Several studies have established that administration of such antagonists, such as aprepitant, together with the 5-HT3 receptor antagonist, lessens chemotherapy-induced nausea and vomiting in patients who are receiving emetogenic chemotherapy during the first 120 hours after initiation of chemotherapy.[30] Although aprepitant was not used commonly during the study period in our country, it is now used widely in clinical practice. Although the exact functional association between ghrelin and NK-1 receptor still is under investigation, their synergistic effect would be novel, and it would be interesting to resolve this issue in a clinical setting in the near future.

Exogenous ghrelin, as expected, successfully increased oral intake and nutritional status and also maintained QoL during chemotherapy. However, our ultimate objective is to ease the completion of chemotherapy and to enhance the overall antitumor effect. In this study, the required dose modifications in the second cycle of chemotherapy tended to be fewer in the ghrelin group (6 patients; 30%) than in the placebo group (10 patients; 50%). Specifically, modifications in the ghrelin group were because of 3 episodes of neutropenia, 2 episodes of thrombocytopenia, and 1 episode of nephrotoxicity; whereas the reasons for modifications in the placebo group included 6 episodes of neutropenia, 3 episodes of nephrotoxicity, and 1 episode of diarrhea. This suggests that ghrelin can prevent some adverse events directly in addition to its indirect effects through improvement of nutritional status. A larger cohort study is needed to verify this aspect of ghrelin administration.

Another clinical question to be answered is whether nutritional support during chemotherapy should be provided orally or intravenously.[31] Recently, we conducted a randomized trial to address this issue in patients with esophageal cancer who were receiving cisplatin-based chemotherapy. Various adverse effects of the chemotherapy, including hematologic toxicity, were observed less frequently in patients who received forced enteral nutrition than in those who received parenteral nutrition, although their total calorie intake was identical (unpublished data). This observation encourages the clinical application of ghrelin administration, which can physiologically increase oral food intake.

In terms of chemotherapy regimens, for this study, both the ACF regimen and the DCF regimen were used. Recently, intensive chemotherapy protocols involving multiple drugs are in fashion; however, to use such regimens, the adverse effects of the regimen components must be adequately managed. An appropriate nutrition supplement through oral food intake will be more important in the future.

In conclusion, the current study demonstrated that short-term administration of exogenous ghrelin at the start of cisplatin-based chemotherapy stimulated food intake and minimized adverse events. We believe that ghrelin administration could increase the efficiency of chemotherapy, and we recommend the use of ghrelin in clinical practice.

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CONFLICT OF INTEREST DISCLOSURES

The authors made no disclosures.

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DISEASES OF THE ESOPHAGUS

Original article

Ten cases of gastro-tracheobronchial fistula: a serious complication after esophagectomy and reconstruction using posterior mediastinal gastric tube

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SUMMARY. **Gastro-tracheobronchial fistula (GTF) is a rare but life-threatening complication specifically observed after esophagectomy and reconstruction using posterior mediastinal gastric tube. Ten cases of GTF were encountered in three hospitals in 2000–2009. Their clinicopathological, surgical, and postoperative care are summarized, together with a review of previously reported cases. GTF was classified as anastomotic leakage (n = 5), gastric necrosis (n = 4), and gastric ulcer type (n = 1). The anastomotic leakage type appeared about 2 weeks (postoperative day [POD]: 8–35) after esophagectomy, was located in the cervical or higher thoracic trachea. Breathing and pneumonia were controlled by tracheal tube placed in the distal of fistula. The gastric necrosis type was noted in patients who developed necrosis of the upper part of the gastric tube and abscess formation behind the tracheal wall, at POD 20–36 around the carina, the site of pronounced ischemia. Due to the large fistula around the carina, emergency surgery with muscle patch repair was frequently required for the control of aspiration pneumonia. Patients of the gastric ulcer type had peptic ulcer in the lesser curvature of the gastric tube, which perforated into the right bronchus long after surgery (POD 630). With respect to tracheobronchial factors, preoperative chemoradiation (three cases) and pre-tracheal node dissection (three cases) tended to increase the risk of GTF. Closure of GTF by surgery (muscle patch repair) was successful in four cases and by nonsurgical treatment in three cases. In one case, stable oral intake was achieved by bypass operation without closure of GTF. Hospital death occurred in three cases. Understanding the pathogenesis and treatment options of GTF is important for surgeons who deal with esophageal cancer.**

KEY WORDS: **esophageal cancer, esophagectomy, gastro-tracheobronchial fistula, reconstruction.**

INTRODUCTION

Surgery is the most reliable curative treatment for esophageal cancer. However, subtotal esophagectomy with gastric tube reconstruction is extremely invasive surgery, and is associated with high morbidity and mortality rates. Gastro-tracheobronchial fistula (GTF) is a rare but serious complication after subtotal esophagectomy with gastric tube reconstruction.[1] Patients with GTF often develop severe aspiration pneumonia leading in some cases to respiratory distress. Systemic condition is often critical since GTF sometimes occurs after other postoperative complications such as anastomotic leakage, mediastinal abscess, and tracheal ischemia. GTF is specifically observed in patients with posterior mediastinal reconstruction but not in retrosternal or subcutaneous reconstruction. Retrosternal reconstruction is the most commonly employed, while the use of posterior mediastinal route with cervical or high thoracic anastomosis has increased and become more popular, probably because of the favorable swallowing function and low risk of anastomotic leakage.[2–5] The incidence of GTF is likely to increase in the future with the increased selection of posterior mediastinal route reconstruction.

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The pathogenesis and treatment of GTF varies from one case to another and are often difficult to generalize. The pathogenesis of GTF should be based on the problem of gastric tube and/or tracheobronchial tree. For example, anastomotic leakage, gastric tube necrosis, and peptic ulcer are complications related to the gastric tube, while ischemia, surgical injury, and irradiation relate to the tracheobronchial tree. Treatment of GTF is difficult, since repair of both the airway and digestive tract is required. Surgical treatment for GTF is highly invasive but is sometimes successful. On the other hand, conservative treatment results in the amelioration of GTF in other patients.[6] However, a subgroup of patients is often refractory to both surgical and conservative treatment. To understand the complication of GTF, we need to investigate more cases and analyze the data systematically.

We experienced 10 cases of GTF during this decade; some were successfully treated, and some were not. We describe here their clinical features in detail, and summarize their clinical course. To compare our results with those of other investigators, we collected and analyzed GTF cases reported in the literature. Backed by the results of our study and those of others, we constructed a strategy for the management of GTF.

PATIENTS AND METHODS

We reviewed the records of patients with esophageal cancer who were admitted to three high-volume institutes (Osaka University, Osaka Medical Center for Cancer and Cardiovascular Diseases, and Kinki University) between 2000 and 2009 and found 10 patients who developed GTF postoperatively. In all patients, the diagnosis of GTF was made by an esophagogastric and/or tracheobronchial fiberscope.

We also searched the 2000–2010 PubMed database and found 31 papers published in peer-reviewed journals that described 'esophagobronchial fistula,' 'tracheogastric tube fistula,' 'gastrobronchial fistula,' 'gastric tube-to-tracheal fistula,' 'broncho-gastric fistula,' and 'gastrotracheal fistula' in patients who developed GTF following esophagectomy.

RESULTS

Patient background

This retrospective study covered 603 patients who underwent subtotal esophagectomy with gastric tube reconstruction through posterior mediastinal route during 2000–2009. Nine patients developed GTF postoperatively, thus the incidence of GTF is 1.5% in three institutions. Another patient in another hospital underwent esophagectomy, developed GTF postoperatively, and underwent surgical repair of GTF but was unsuccessful, then was transferred to Osaka University for further management of GTF. Thus, the study included 10 patients. Table 1 summarizes the clinical/surgical data of the 10 patients. Surgery for esophageal cancer was basically identical in all three participating institutions, and included subtotal esophagectomy with two or three field lymph node dissection via right thoracotomy, and reconstruction using gastric tube pull-up through the posterior mediastinal route.[7,8]

The age (mean, 62.6 years) and gender (M : F 9 : 1) of the subjects were not different from the esophageal cancer patients registered in the nationwide registry.[9] Patients with upper thoracic tumor ($n = 3$) commonly underwent pre-tracheal lymph node dissection. Advanced disease was common in the cohort, based on the TNM classification (6th version).[10] Three patients underwent preoperative chemoradiotherapy, while three others received chemotherapy preoperatively. None of the patients received postoperative chemo or radiotherapy.

Classification of GTF

The most significant and consistent finding in GTF patients was problems related to the gastric tube, which were classified as anastomotic leakage ($n = 6$), gastric tube necrosis ($n = 3$), and peptic ulcer of the stomach ($n = 1$). These problems were diagnosed by esophagogastric fiberscopy performed after GTF formation. Gastric tube necrosis was caused by insufficient blood supply localized to the tip and lesser curvature of the gastric tube.

The date of GTF formation was clinically evident since severe cough and pneumonia-related symptoms appeared suddenly. The interval between esophagectomy and the development of GTF ranged from 8 to 47 days (mean, 27 days) in patients with anastomotic leakage or gastric tube necrosis, while that in patients with peptic ulcer-related GTF was longer (630 days). On the other hand, the date of occurrence of anastomotic leakage and/or gastric necrosis could not be identified in some cases. In other patients (1, 2, 3, 4, 8, and 9), the date of anastomotic leakage was clinically apparent (day 7 to 11, average day 9). The latency between anastomotic leakage and GTF formation was approximately 2 weeks. This finding suggests that the tracheobronchial wall was damaged secondarily by the effect of the anastomotic leakage (R1-Q1). Figure 1 shows schematically the location of each GTF in the airway. In all cases, the fistula was located in the posterior, i.e. membranous, wall of the tracheobronchial tree. The location of GTF in the tracheobronchial tree correlated with the type of gastric abnormality; five out of six patients with anastomotic leakage type developed GTF in the cervical or the thoracocervical junction part of the trachea.

Table 1 Clinicopathosurgical features of 10 patients with gastro-tracheobronchial fistula

No.	Age (years)/sex	cStage	Location	LN dissection	Preoperative treatment	Days to GTF	Type of gastric insufficiency	Site of fistula in the airway	Primary treatment	First operation	Reconstruction	Outcome at hospital discharge
1	60/M	T3N1M0	Lt	2-field	–	8	AL	High trachea	Decompressive tube	GR+muscle patch	Rt hemicolon	Recovery POD86
2	61/M	T2N0M0	Ut	3-field	CRT (60 Gy)	47	AL	High trachea	Tracheal tube	None	NR	Recovery POD191
3	62/M	T4N1M1lym	Mt	3-field	Chemotherapy	16	AL	High trachea	Tracheal tube	None	NR	Recovery POD55
4	68/M	T1N0M0	Mt	2-field	–	18	AL	High trachea	Decompressive tube	None	Rt hemicolon	Recovery POD253
5	61/M	T3N1M0	Ut	3-field	CRT (60 Gy)	38	AL	High trachea	Tracheal tube	None	NP	Death POD247
6	72/M	T1N0M0	Mt	2-field	CRT (60 Gy)	35	AL	Low trachea	Operation	GR+muscle patch	Pedicled jejunum	Death POD1074
7	64/M	T3N1M1lym	Lt	3-field	Chemotherapy	20	Gastric necrosis	Low trachea	Operation	GR+muscle patch	Rt hemicolon	Recovery POD377
8	61/F	T2N0M0	Mt	3-field	–	24	Gastric necrosis	Low trachea	Operation	GR+muscle patch	Rt hemicolon	Recovery POD205
9	55/M	T3N1M0	Lt	3-field	Chemotherapy	36	Gastric necrosis	Low trachea	Operation	EY+GT transection	NP	Death POD64
10	60/M	T3N1M0	Ut	2-field	–	630	Gastric ulcer	Right bronchus	Operation	EY+GT transection	Pedicled jejunum	Recovery POD240

AL, anastomotic leakage; EY, esophagotomy; CRT, chemoradiotherapy; F, female; GR, gastric resection; GT, gastric tube; GTF, gastro-tracheobronchial fistula; LN, lymph node; Lt, lower third of the trachea; M, male; Mt, middle third of the trachea; NP, not performed; NR, not required; Rt, right; Ut, upper third of the trachea.

Fig. 1 Location of GTF in the tracheobronchial tree. Each number indicates the location of GTF of the patients listed in Table 1. TGF were classified as anastomotic type (from 1 to 6), gastric necrosis type (7, 8, and 9) and gastric ulcer type (10).

The remaining patient developed leakage followed by mediastinal abscess with subsequent development of GTF just above the carina. In patients with gastric tube necrosis, a wide area of the membranous trachea, extending from the cervical region to near carina, was exposed to the gastric juice (Fig. 2a), with the GTF was observed in the distal trachea and carina area. In patients with peptic ulceration, GTF developed in the right bronchus, which is in close proximity to the lesser curvature of the body of the gastric tube where peptic ulcer is frequently observed (Fig. 2b).

Treatment and outcome of GTF

Treatment of GTF varied according to the type of gastric disease. In the majority of patients with the anastomotic leakage type (Patients 2, 3, 4, and 5), leakage of gastric fluid into the airway was blocked by using the cuff of a tracheal tube placed in the distal part of the fistula. Notably in two of these patients, the fistula was small and closed spontaneously along with the cessation of leakage from the esophagogastric anastomsis. In Patient 1, aspiration pneumonia was managed successfully by placing a decompressive tube in the gastric tube; however, this was unsuccessful with persistence of the fistula (approximately

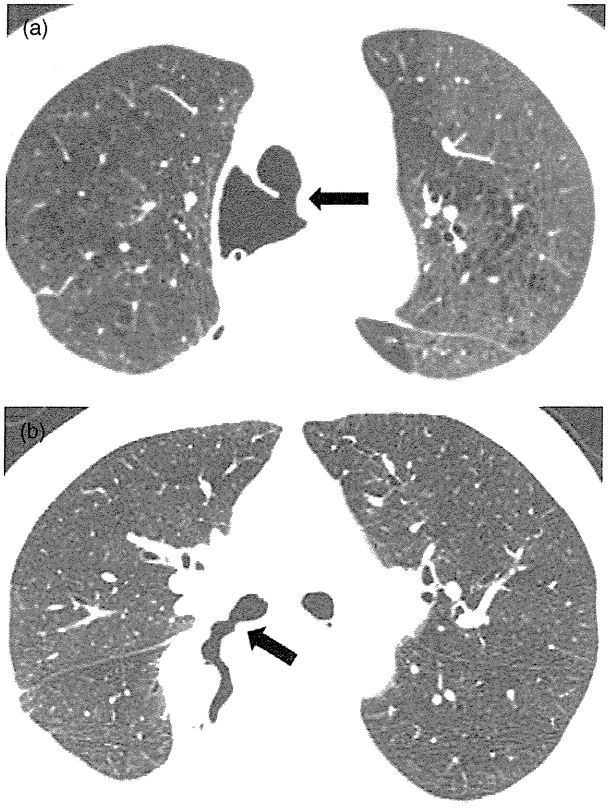

Fig. 2 CT scan features of GTF. The presence of GTF was confirmed by fiberscopy, while the location was well recognized on the CT scan. (a) TGF in the trachea near the carina in Patient 8. (b) A huge TGF in the right bronchus that had been treated surgically in another hospital, but the treatment was unsuccessful (Patient 10).

7 mm in diameter). Therefore, surgical repair was performed after improvement of pneumonia. In Patient 6, the location of the GTF was close to the carina, and prevention of aspiration was not possible by using the tracheal tube. Accordingly, this patient underwent emergency operation to close the GTF. In the gastric necrosis type of GTF, the fistula was larger (>1 cm), the general condition was poorer, and aspiration pneumonia was more severe and critical than the other types. In addition, the huge fistula located near the carina could not be blocked by the tracheal tube. All three patients with the gastric necrosis type of GTF underwent emergency operation on the day of diagnosis. Two of them (Patients 7 and 8) underwent surgical repair of GTF, while the other underwent palliative surgery to reduce gastric content aspiration. In the gastric ulcer type (Patient 10), surgical closure using intercostal muscle had been tried in another hospital; however, this treatment was not successful, resulting in increase in the size of the GTF. This patient suffered from persistent aspiration pneumonia due to the huge GTF when admitted to our hospital. Palliative surgical treatment for GTF was attempted for this patient.

Taken together, four patients (Patients 1, 6, 7, and 8) underwent surgical repair of GTF, consisting of removal of the gastric tube and insertion of a muscle patch to the fistula using a pedicle of the pectoralis major muscle in one patient and latissimus dorsi muscle in three patients (Fig. 3). The latter operative technique, which could be the most promising though highly invasive, requires re-thoracotomy and synechiotomy of the thoracic cavity. Surgical repair of the GTF was abandoned in two patients (Patient 9 and 10) who received palliative surgical treatment to improve the aspiration pneumonia. This consisted of esophagostomy and transection of the gastric tube at the pyloric portion. Aspiration of saliva was prevented by the former, and the reflux of bile and pancreatic fluid was prevented by the latter. Gastric fluid hypersecretion was minimized by proton pump inhibitor, and a drainage tube was placed in the remnant gastric tube.

Six patients underwent re-reconstruction of the esophagus using jejunal pedicle in two patients and right-side pedicle of the colon in four patients. Patient 10 underwent reconstruction using a jejunal pedicle despite the persistent GTF and intrathoracic gastric tube. Among the 10 patients, one died because of pneumonia at 64 days after surgery, and two others died at 274 and 1,074 days after surgery because of cancer recurrence.

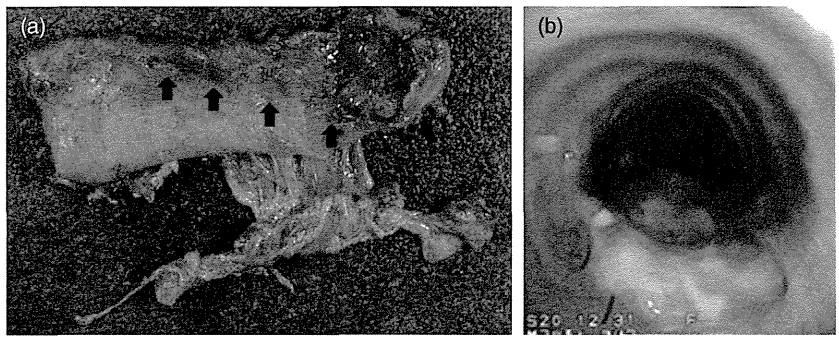

Fig. 3 Surgical treatment of GTF. Patient 8 underwent emergency operation including removal of the gastric tube and closure of GTF by muscle patch. (a) Removed stomach showed necrosis of the upper and lesser curvature parts of the tube (arrows). (b) Postoperative fiberscopy showed a large defect in the posterior tracheal wall. This was covered with muscle patch using latissimus dorsi muscle.

Literature review of patients with GTF

Among the 38 cases of GTF reported in the literature, 15 patients had no problems with the gastric tube. Instead, the pathology of GTF included injury by the tracheal tube and balloon dilatation for anastomotic stricture. In 12 cases, leakage from the upper part of the gastric tube was apparent on the esophagogram; however, we could not determine whether it was anastomotic leakage or gastric tube necrosis, since no fiberscopy was performed. In 11 patients, peptic ulcer in the gastric tube was confirmed by fiberscopy or gastrography. For this reason, the GTF was classified into three types: gastric leakage, peptic ulcer, and others (Table 2).

The interval from esophagectomy and development of GTF was different among the reported cases and the three groups. Consistent with our cohort, GTF occurred at a mean of 20.4 days after surgery in the gastric leakage group and 1,573 days after surgery in the peptic ulcer group. The distribution pattern of the GTF in the airway was also similar to that of the cohort; GTF associated with peptic ulcer was observed mostly in the right bronchus, while that in the gastric leakage group was located at a higher position of the trachea.

The treatment and outcome of GTF were not always identical to those of the cohort. In 12 patients with the gastric leakage type, curative treatment with muscle flap was performed in eight patients, and only one patient died of GTF. In the 11 patients with peptic ulcer type, muscle flap was less frequently performed (four patients only), while conservative treatment was successful in three patients.

Table 2 Summary of 38 cases of gastro-tracheobronchial fistula (GTF) reported in the literature

	Gastric leakage (n = 12)	Peptic ulcer (n = 11)	Other/ unknown (n = 15)
Days to GTF median (range)	14 (8–60)	1,460 (60–4015)	42 (10–2190)
Perioperative radiation			
Performed	3	7	4
Not performed	6	4	8
Unknown	3	0	3
Site of fistula in the airway			
High trachea	2	0	5
Low trachea	4	2	1
Right bronchus	4	8	6
Left bronchus	1	1	2
Unknown	1	0	1
Treatment			
Surgical treatment	11	8	10
(Muscle patch)	(8)	(4)	(3)
Endoscopic treatment	1	0	2
Conservative	0	3	3
Prognosis			
Recovery	10	7	14
Death	1	4	0
Unknown	1	0	1

DISCUSSION

There is general agreement that GTF is one of the most difficult complications after esophagectomy and reconstruction with posterior mediastinal gastric tube. Development of GTF compromises the integrity of the airway and digestive tract, resulting in severe aspiration pneumonia. This study is the largest study of GTF, including 10 managed at our hospitals and review of 34 reported cases, which comprehensively analyzed the pathogenesis and discussed the treatment of GTF.

Although both gastric and tracheobronchial factors are involved in the pathogenesis of GTF, gastric factors seem to be more important than tracheobronchial factors based on the analysis of our patients. For this reason, we focused on the gastric factors and classified GTF as anastomotic leakage, gastric necrosis, and gastric ulcer types. The majority of the gastric necrosis type showed that necrosis was limited to the tip or lesser curvature of the stomach. Such partial necrosis was difficult to identify on the esophagogram, though this was possible by fiberscopy. In our patients, fiberscopy was conducted routinely when anastomotic leakage was suspected, while it was seldom performed in the reported cases. Therefore, we combined the anastomotic leakage and necrosis types into the gastric leakage type in the analysis of the previously reported GTF (R1-Q2). The blood supply to the gastric tube is a major risk factor for anastomotic leakage and/or gastric tube necrosis. Previous studies reported that subtotal gastric tube displayed better blood supply and less anastomotic leakage than narrow gastric tube.[11] It is also reported that the presence of vessel anastomosis between the right and left gastroepiploic arteries showed better blood flow at the tip of the gastric tube than without such anastomosis.[12]

The location of GTF in the tracheobronchial tree tended to be determined by the type of gastric disorder. That is, the anastomotic leakage type was frequently associated with a high tracheal lesion, while the gastric necrosis was associated with a low tracheal lesion and gastric ulcers in the right bronchus. Another characteristic was a huge difference in the latency to GTF formation after esophagectomy. Both the anastomotic leakage and gastric necrosis types occurred within 1 month after surgery, while the gastric ulcer type occurred more than 1 year after surgery. In the cases reported in the literature, one third of GTF could not be categorized into any of the classifications used in our study, and the pathology insults included balloon dilation for anastomotic

stricture,[6,13] leakage of tracheotoma,[14] and compression necrosis by tracheal tube cuff.[15]

The tracheobronchial factors also played an important role in the development of GTF. In general, damage of the tracheobronchial tree, also known as tracheobronchial lesion (TBL),[16,17] is a critical postoperative complication of esophagectomy, and several studies investigated the clinical profile of TBL. These reports identified several risk factors of TBL, including perioperative irradiation, peritracheal lymph node dissection, and ischemia associated with resection of bronchial and inferior thyroid arteries. In addition, Bartels *et al.* reported that TBL caused by ischemia was frequent around the carina, while TBL caused by other reasons, for example, injury by the tracheal tube or surgical manipulation, was frequent in higher tracheal lesion.[1] They also reported that the incidence of TBL was 3.9% (31/785) while that of GTF was 0.8%, i.e. four among 501 cases of posterior mediastinal reconstruction. The incidence in our patients was 1.5% (9/603). This value was slightly higher than their series, probably because our cases included more of mediastinal lymph node dissection via right thoracotomy and salvage surgery. Salvage surgery after definitive chemoradiotherapy is novel issue for esophageal surgeons to conquer the associated high morbidity and mortality. In this regard, Tachimori *et al.* reported that the incidence of both trachea and gastric tube necrosis is 3% after salvage surgery.[18]

Once GTF is diagnosed, control of aspiration pneumonia is the most important issue. In the anastomotic leakage type, since GTF was frequently located in the cervical or higher thoracic trachea, aspiration of gastric content could be blocked by the cuff of tracheal tube. Such cases required elective surgery. In the gastric ulcer type, GTF occurred long after surgery when systemic condition was much better than the postoperative period. In addition, aspiration pneumonia was localized since GTF was often located in the distal end of the tracheobronchial tree, especially in the right bronchus. Thus, in the majority of cases, elective treatment was possible simply by suspending oral intake. The most critical aspiration pneumonia was observed in the gastric necrosis type for the following reasons. GTF frequently developed around the carina where blockade of aspiration was not possible by the cuff of the tracheal tube. GTF in this lesion was often large in size and caused by local ischemia as described above. GTF was frequently preceded by septic state due to gastric necrosis and subsequent mediastinal or thoracic abscess. Thus, emergency operation is basically indicated for the gastric necrosis type of GTF.

GTF requires surgical repair of both the airway and digestive tract. However, in many cases, simultaneous repair was not possible because of excess surgical stress, and closure of the defect of the airway has more priority than that of the digestive tract. Pedicled muscle patch, using latissimus dorsi muscle,[14,19–21] pectoralis major muscle,[13,22,23] and intercostal muscle, along with removal of gastric tube, seems to be the most reliable procedure for recovering the airway integrity. Digestive tract repair was secondary and electively performed using organs other than the stomach including pedicled jejunum and right side colon. (R1-Q5) When the oral remnant esophagus is short, the right side colon would be preferable. On the other hand, although surgery is primarily indicated for the repair of GTF, successful conservative treatments have been conducted and reported for the anastomotic and gastric ulcer type GTF. We experienced spontaneous closure and, repair of GTF using covering stent or fibrin glue has been reported in the literature.[15,22,24,25] (R1-Q3Q4) If the GTF is small (e.g. pin hole) and gastric wall is not necrotic or ischemic, spontaneous closure of GTF might be expected. However, when the GTF is relatively large, it is frequently accompanied with gastric necrosis or abscess behind the tracheal wall and thus spontaneous closure of GTF should not be expected. In two of our patients, surgical closure of GTF was not indicated because of poor systemic condition (Patient 9) and surgical problem, i.e. failure of the first surgical repair (Patient 10). In these two patients, we were able to control aspiration pneumonia by palliative treatment including esophagotomy, gastric tube transaction, and proton pump inhibitors as described above.

In general, the prognosis of GTF was poor, with 3/10 (30%) mortality rate in our patients and 5/38 in the reported cases. The most common causes of death are prolonged pneumonia and respiratory failure.

In conclusion, management of GTF including surgical repair requires the highest knowledge and skill. Our experience and the review of reported cases here should help understand this rare but often lethal postoperative complication.

Acknowledgment

The authors declare no conflict of interest.

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Clinical Cancer Research

Let-7 Expression Is a Significant Determinant of Response to Chemotherapy through the Regulation of IL-6/STAT3 Pathway in Esophageal Squamous Cell Carcinoma

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Abstract

Purpose: Cisplatin-based chemotherapy is widely used for esophageal cancer, sometimes in combination with surgery/radiotherapy, but poor response to chemotherapy is not uncommon. The aim of this study was to examine whether miRNA expression is useful to predict the response to chemotherapy in patients with esophageal cancer.

Experimental Design: Using pretreatment biopsy samples from 98 patients with esophageal cancer who received preoperative chemotherapy, we measured the expression level of several miRNAs whose expression was altered in cisplatin-resistant esophageal cancer cell lines compared with those parent cell lines and examined the relationship between the miRNA expression and response to chemotherapy. *In vitro* assays were conducted to clarify the mechanism of miRNA-induced changes in chemosensitivity.

Results: The expression levels of 15 miRNAs were altered in cisplatin-resistant cells. Of these, low expression of let-7b and let-7c in before-treatment biopsies from 74 patients of the training set correlated significantly with poor response to chemotherapy, both clinically and histopathologically. Low expression of let-7c also correlated with poor prognosis ($P = 0.032$). The relationship between let-7b and let-7c expression and response to chemotherapy was confirmed in the other 24 patients of the validation set. In *in vitro* assay, transfection of let-7c restored sensitivity to cisplatin and increased rate of apoptosis after exposure to cisplatin. Let-7c directly repressed cisplatin-activated interleukin (IL)-6/STAT3 prosurvival pathway.

Conclusions: Let-7 expression in esophageal cancer can be potentially used to predict the response to cisplatin-based chemotherapy. Let-7 modulates the chemosensitivity to cisplatin through the regulation of IL-6/STAT3 pathway in esophageal cancer. *Clin Cancer Res; 18(18); 5144–53. ©2012 AACR.*

Introduction

Despite recent advances in surgical techniques and perioperative management, the prognosis of patients who undergo surgery alone for esophageal cancer remains poor (1). Neoadjuvant chemotherapy or chemoradiotherapy followed by surgery has emerged as a promising strategy for advanced esophageal cancer and in fact, good responders to such preoperative therapy show better survival (2, 3). However, the reported response rate to cisplatin-based chemotherapy, which is widely used for esophageal cancer, is only modest, ranging from 25% to 48% (4–7) and nonresponders likely receive no survival benefit (8). The ability to predict the response to chemotherapy before treatment should limit the application of chemotherapy to selected patients who are likely to show some benefits, and allow tailoring such therapy to the individual patient with esophageal cancer.

miRNAs are noncoding RNAs of approximately 22 nucleotides in size and act by repressing the translation of target mRNA by binding to the 3′-untranslated region of those mRNAs (9). miRNAs exist stably in various tissues and play pivotal roles in differentiation and development (10). In addition, aberrant expression of miRNAs is reported in various types of cancers. In esophageal cancer, miR-21 and miR-93 are reported to be upregulated, whereas miR-375, miR-27b, miR-203, miR-205, and let-7c are downregulated (11, 12). Recent studies also showed the involvement of several miRNAs in resistance to anticancer treatment including chemotherapy and radiotherapy. Giovannetti and colleagues (13) reported that overexpression of miR-21was associated with poor outcome in gemcitabine-treated patients with pancreatic cancer. In our previous study using residual tumor after chemotherapy, we showed the involvement

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Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

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