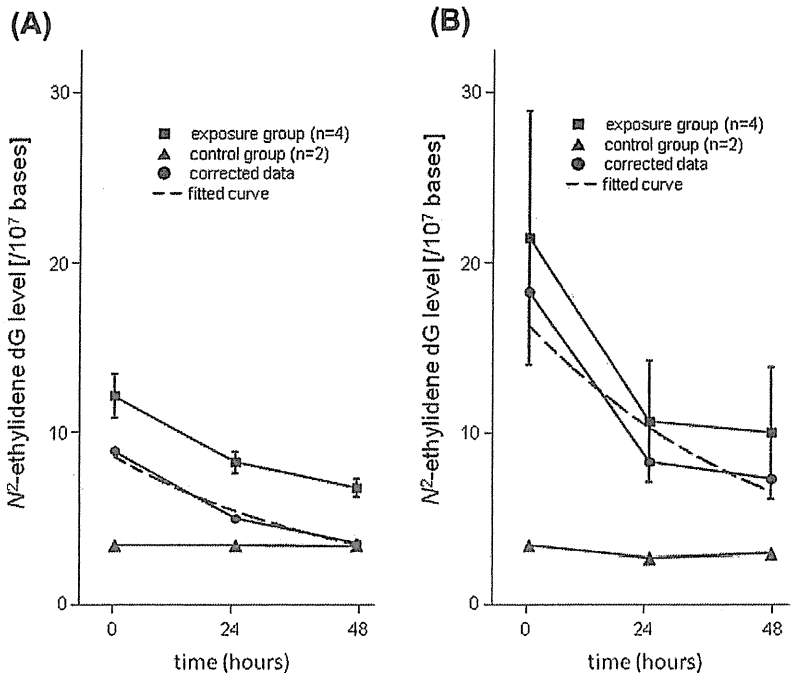

Fig. 4. Half-life of N^2-ethylidene-dG in vitro. (A) Adduct levels after 1 h exposure to 0.01% (1.8 mM) acetaldehyde (AA). (B) Adduct levels after 2 h exposure to 0.01% (1.8 mM) AA. The mean levels are shown in the figure at each time. The data points represent the corrected data, which were calculated by subtracting the mean control level ($n = 2$) from the mean level in the exposed cells ($n = 4$) at each time. The two fitted curves (dashed lines) could be represented as an exponential approximation expressed mathematically as follows: $y = 8.50e^{-0.02x}$ ($R^2 = 0.98$) in the 1 h exposure group and $y = 16.2e^{-0.02x}$ ($R^2 = 0.85$) in the 2 h exposure group. The same half-life ($t_{1/2}$) of about 35 h ($t_{1/2} = \log_e 2/0.02 = 0.693/0.02 = 34.65$) was derived from these independent experiments. The error bars represent the standard deviation.

experiment. The first limitation is that the AA concentration of 0.01% (1.8 mM) used in our experiment is much higher than in the in vivo condition. In humans, the ability to metabolize alcohol differs considerably between individuals and is dependent on the activity of aldehyde dehydrogenase-2 (ALDH2). After ingestion of 0.4 g/kg ethanol, the blood AA level scarcely increases in people homozygous for ALDH2*1, but it increases to 23.4 μM in people heterozygous for ALDH2*1/*2 and further to 79.3 μM in those homozygous for ALDH2*2 [11]. We confirmed that HL60 cells have fully active ALDH2 activity (homozygous ALDH2*1 genotype), and we could not avoid having to use a high concentration of AA to detect the DNA adduct in vitro. Further study using another cell line with the low-activity ALDH2*2 allele is desirable. Alcohol-dependent increases in N^2-ethylidene-dG levels can be detected even in mice with the homozygous ALDH2*1 genotype [8,9], suggesting that the stability of AA-derived DNA adducts is different in vivo and in vitro.

The second limitation of our study is that we detected only one kind of adduct, N^2-ethylidene-dG. To ensure accurate measurement, all AA-derived DNA adducts should be analyzed. Matsuda et al. developed a sensitive method using LC/MS/MS to quantitatively detect other AA-derived DNA adducts including α-methyl-hydroxy-1, N^2-propano-2-dG (α-Me-γ-OH-PdG), N^2-(2,6-dimethyl-1,3-dioxan-4-yl)-2-dG (N^2-Dio-dG), and 8-oxo-7,8-dihydro-2-dG (8-oxo-dG) [8–10]. However, in the case of α-Me-γ-OH-PdG and 8-oxo-dG, neither alcohol-dependent nor ALDH2 genotype-dependent increases in adduct levels were observed [8,9]. N^2-Dio-dG has not been detected in human DNA samples [10]. Therefore, it is reasonable to expect that AA-derived DNA adducts can be represented by N^2-ethylidene-dG.

The third limitation of our study is that only one exposure of HL60 cells to AA was performed in our experiments, and this experimental model does not mimic the condition of chronic alcohol consumption. Repeated or continuous exposure to AA may lead to the extension of the $t_{1/2}$ of AA-derived DNA adducts. Conversely, we were surprised by the long $t_{1/2}$ of AA-derived DNA adducts (35 h) even under the conditions of rapid AA metabolism in this study (i.e., full ALDH2 activity and transient exposure to AA).

In conclusion, we examined the stability of an AA-derived DNA adduct in vitro. The long $t_{1/2}$ of the AA-derived DNA adduct (35 h), even under the fully active ALDH2 condition, suggests that exposure to AA by daily alcohol consumption may cause DNA damage and may increase the risk of alcohol-related carcinogenesis.

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ORIGINAL ARTICLE: Clinical Endoscopy

Usefulness of endoscopic radial incision and cutting method for refractory esophagogastric anastomotic stricture (with video)

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Kyoto, Japan

Background: There is no effective treatment for gastroesophageal anastomotic strictures that are refractory to repeated endoscopic balloon dilation (EBD). However, EBD is still selected worldwide to manage such refractory strictures. To relieve the symptoms of dysphagia and keep a wide lumen, we developed a new incisional treatment, radial incision and cutting (RIC).

Objective: To evaluate the efficacy and safety of the RIC method for the treatment of refractory anastomotic strictures.

Design: Retrospective cohort study.

Setting: National Cancer Center and University Hospital.

Patients: This study involved 54 consecutive patients with refractory anastomotic stricture after esophagogastric surgery.

Intervention: RIC.

Main Outcome Measurements: The safety and clinical success of RIC and the long-term patency after RIC compared with those of continued EBD.

Results: The median procedure time of RIC was 14 minutes (range, 4–40 minutes). No serious adverse events associated with RIC were observed. Immediately after RIC, 81.3% (26/32) of patients were able to eat solid food without symptoms of dysphagia. As a short-term effect, the dysphagia improved after RIC in 93.8% (30/32) of the patients. As a long-term effect, 63% (17/27) and 62% (13/21) of patients were able to eat solid food 6 and 12 months after RIC, respectively. The 6-month and 12-month patency rates were significantly different between the RIC group and the continued EBD group (65.3% vs 19.8%, $P < .005$; 61.5% vs 19.8%, $P < .005$).

Limitations: Nonrandomized retrospective study.

Conclusions: RIC is an effective and safe method. The demonstration of the validity of this method may place RIC as a new medical treatment for patients with refractory stricture after surgical resection for esophagogastric diseases. (Gastrointest Endosc 2012;75:965-72.)

Benign anastomotic strictures of the esophagus after surgical resection are unfortunately encountered as the result of treatment for esophagogastric diseases. Dysphagia caused by anastomotic stricture impairs the quality of life of patients drastically. Usually, endoscopic balloon dilation (EBD) or rigid dilation relieves the symptoms of

Abbreviations: EBD, endoscopic balloon dilation; IT, insulated tip; RIC, radial incision and cutting.

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dysphagia in the majority of cases of anastomotic esophageal stricture. In general, 1 to 3 dilations are needed to relieve the dysphagia caused by simple, short strictures that allow the passage of an endoscope of normal diameter (≥10 mm). However, some patients have severe dysphagia because of strictures that are refractory to repeated dilations. Nevertheless, EBD or rigid dilation is still selected worldwide to manage such refractory strictures.

Simmons and Baron[1] and Hordijk et al[2] reported the usefulness of electrocautery treatment for anastomotic esophageal strictures that are refractory to repeated EBD or Savary bougienage. However, the superiority of electrocautery treatment as a second-line treatment for refractory anastomotic strictures vs continued EBD or rigid dilation has not been demonstrated.

Anastomotic strictures that are refractory to repeated dilation are associated with severe fibrotic changes, after which dilation seems to be ineffective. To keep a wider lumen, we originally developed a new electrocautery treatment: the radial incision and cutting (RIC) method.[3] In the RIC method, radial incisions are performed with an insulated-tip (IT) knife, followed by cutting away of the fibrotic tissue between the incisions.

In this study, we evaluated the efficacy, safety, and long-term patency of the RIC method as a second-line treatment after repeated EBD for refractory anastomotic strictures. We also compared the effectiveness of the RIC method with that of the continued repeated EBD approach.

PATIENTS AND METHODS

Patients

From May 2006 to March 2011, 32 consecutive patients with refractory anastomotic stricture after esophagogastric surgery were treated with RIC and were followed up at the National Cancer Center Hospital East and the Kyoto University Hospital. Refractory esophagogastric stricture was considered when the stricture could not be improved to a diameter larger than 10 mm and 3 or more sessions of EBD (CRE balloon dilator, Boston Scientific, Natick, MA) with at least 1 week of interval had been administered.

The RIC procedure was indicated for patients with refractory anastomotic stricture who had received at least 3 repeated sessions of EBD and for whom the symptoms of dysphagia were not relieved. To investigate the efficacy and safety of the RIC method, we compared these findings with the results obtained in another 22 consecutive patients with refractory anastomotic stricture after esophagogastric surgery performed at the Kyoto University Hospital during the same period, who received more than 4 repeated EBD sessions with at least 1 week of interval at their request.

Written informed consent was obtained from all of the patients, and this study was approved by the local ethics committees.

Take-home Message

- The radial incision and cutting method for refractory anastomotic strictures resulted in rapid improvements in patency and food intake among patients.
- The 6-month patency rate was significantly higher with the radial incision and cutting method than with continued balloon dilation, although this study did not include a direct head-to-head comparison.

Participating endoscopists

Both RIC and EBD procedures were performed by 3 endoscopic specialists (M.M., Y.E., and T.Y.) accredited by the Japan Gastroenterological Endoscopy Society. They had sufficient skills with both endoscopic submucosal dissection with IT knife and EBD.

RIC procedure

All of the patients received 17.5 to 35 mg pethidine hydrochloride to reduce the discomfort associated with the RIC procedure. All RIC procedures were performed by using direct visualization. RIC was carried out as follows (Fig. 1) (Video 1, available online at www.giejournal.org): (1) the stricture area was incised radially by using an IT knife endoscopically; (2) the virtual line that connects the esophageal lumen on the oral side and the lumen on the anal side was assumed, and an incision was performed along this line; (3) the incision area was sliced off with an IT knife; and (4) after RIC, preventive EBD was performed repeatedly at the frequency of once per week, to maintain patency until the cutting surface became a scar.[4] The details of the preventive EBD have been described previously.[4]

EBD procedure

All of the patients received 17.5 to 35 mg pethidine hydrochloride to reduce the discomfort associated with the EBD procedure. All EBD procedures were performed by using direct visualization and fluoroscopic monitoring. We used a multidiameter CRE balloon dilator (Boston Scientific) and performed EBD according to the manufacturer's procedure guidelines. Three CRE balloon dilators of different sizes (10-12 mm, 12-15 mm, and 15-18 mm) were used, according to the severity of the stricture. A single balloon was used in each EBD session. The selection of balloon size was strictly managed as follows: a 15- to 18-mm balloon was used for strictures larger than 5 mm and smaller than 10 mm in diameter; a 12- to 15-mm balloon was used for strictures larger than 2 to 3 mm and smaller than 5 mm in diameter; and a 10- to 12-mm balloon was used for pinhole strictures.

Evaluation of dysphagia before and after RIC

We evaluated the grade of the dysphagia symptoms before and after RIC by using the dysphagia score:[5] 0, able

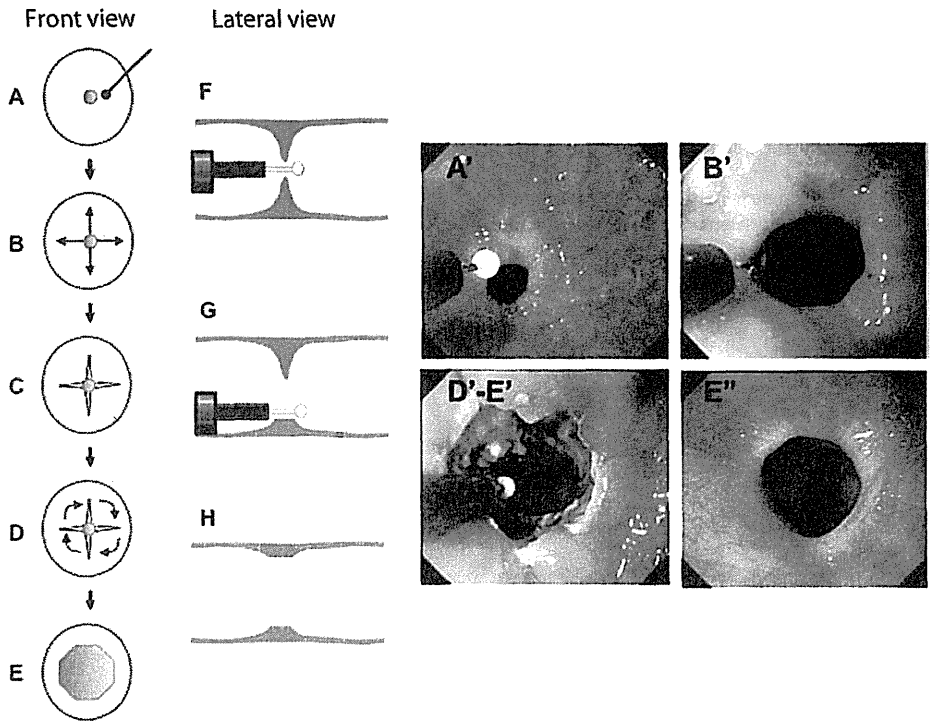

Figure 1. Radial incision and cutting (RIC) procedure. **A, B, C, D, E,** Schematic front views of stricture site. **F, G, H,** Schematic lateral views of stricture site. First, the stricture site was radially incised with an insulated-tip knife (**A, A', F**). The incision was performed to achieve sufficient depth, which was defined as the virtual line that connects the level of the esophageal lumen on the oral and anal sides of the stricture site (**B, C, F, G**). Second, each part of the stricture site located between the adjacent incisions was sliced off (**D, D', E, E', H**). After the RIC procedure, the wounded surface of the stricture site was carefully observed for the presence of any bleeding or perforation (**E, D', E'**). After healing of the RIC-treated site, a wider lumen was obtained (**E"**).

to eat a normal diet; 1, unable to swallow certain solids; 2, able to swallow semisolid foods; 3, able to swallow liquids only; and 4, unable to swallow liquids.

Evaluation of the diameter of the stricture before and after RIC

The diameter of strictures was categorized as follows: (1) an endoscope with a size of 10 mm could pass through the stricture; (2) from 5 mm to smaller than 10 mm; (3) from 2 mm to smaller than 5 mm; and (4) smaller than 2 mm (this stricture size was measured based on contrast to the tip [2 mm] of the IT knife).

Definition of treatment failure and indication of re-RIC treatment

Failure of the RIC or repeated EBD procedure was defined as the inability to pass a standard endoscope with a diameter of 10 mm or larger (Q240, 1T240, H260, and H260Z; Olympus Medical Systems, Tokyo, Japan) through the stricture site after RIC and as the presence of dysphagia with a score higher than 2.

We performed additional RIC treatment (re-RIC) when patients had recurrent symptoms of dysphagia (dysphagia score and the diameter of the stricture met the criteria of treatment failure) or when patients requested re-RIC for further improvement of discomfort during food intake not for the relief of dysphagia.

Statistical analysis

The absolute and relative frequencies of qualitative variables were calculated for each group. The continuous variables were expressed as medians and ranges. Continuous data were compared with the Mann-Whitney U test. Pearson's χ^2 test or Fisher's exact test was used to analyze categorical data, to compare proportions. All P values were 2-tailed, and a P value $<.05$ was considered significant. The cumulative patency of the stricture was measured from the date of the first RIC in the RIC group and the fourth EBD in the continued EBD group to the earliest treatment failure and was estimated with the Kaplan–Meier method, comparisons being made with the log-rank test. Statistical analyses were performed with the StatView software, version 5 (SAS Institute Inc., Cary, NC).

RESULTS

Patient characteristics

Patient characteristics are summarized in Table 1. In the RIC group, the median age was 66 years (range, 33-81

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Cytochrome P450 in non-small cell lung cancer related to exogenous chemical metabolism

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1. ABSTRACT

The occurrence of lung cancer is associated with smoking, which exposes smokers to a series of carcinogenic chemicals. CYP (cytochrome P450) usually metabolizes carcinogens to their inactive derivatives, but occasionally convert the chemicals to more potent carcinogens. In addition to the metabolism of carcinogenic compounds, CYP also participates in the activation and/or inactivation of anti-carcinogenic agents, suggesting that the local CYP expression in lung cancer and surrounding tissues could be an important determinant of efficacy of anticancer drugs. Furthermore, CYP19 (aromatase), estrogen synthase P450, expressed in more than 80% of non-small cell lung cancers. It suggests an association between estrogens and cancer development, which makes aromatase an attractive therapeutic target for the treatment of lung cancer. 1α,25-Dihydroxyvitamin D3 has an inhibitory effect on the proliferation of cancer tissues, and is converted to its inactive 24-hydroxylated derivatives by CYP24, which is frequently expressed in lung cancer tissues. Therefore, understanding the CYP expression in tumor tissues is important in developing better therapies for lung cancer, and may lead us to standardized, "tailor-made" therapies for individuals.

2. INTRODUCTION

Lung cancer is the leading cause of cancer mortality in developed countries, including Canada, France, Italy, the UK, the USA, and Japan (1). Lung tumors are classified into two broad classes, namely, small cell lung cancer (SCLC), accounting for about 15% of the cases, and non-small cell lung cancer (NSCLC), which is the most common form of lung cancer, accounting for up to 85% of all cases (2, 3). There are three types of NSCLC: squamous cell carcinoma, adenocarcinoma, and large cell carcinoma (2). Current clinicopathological staging systems have the advantage of standardized criteria for assessing tumor stage. Using the staging systems, the relationship between advancing tumor stage and poor prognosis has been established for NSCLC. However, these staging systems have not led to clear criteria for therapy selection in individual patients with NSCLC. The concept of therapy based on anatomical location, such as staging systems, is poorly associated with the metabolic characteristics of individual tumor tissues (4).

The cytochrome P450 (CYP) family is a multi-gene superfamily comprising constitutive and inducible heme-containing mono-oxygenases (P450), in which the

Table 1. Expression of *CYP* genes in non-small cell lung cancer

Lung Cancer	Tumor tissue	
	Protein	RNA
CYP1A1	WB (Lower in tumor than normal tissue) (12) WB (20%) (15) IHC (44% of adenocarcinoma) (7) IHC (37% of adenocarcinoma) (13)	NB (23%) (14) RTQPCR (16%) (15)
CYP1B	WB (100%) (16) WB (100%) (15) WB (100%) (17) IHC (47%) (17) IHC (49% of adenocarcinoma) (13)	RTQPCR (100%) (16) RTQPCR (80%) (15)
CYP2A6	IHC (46% of adenocarcinoma) (7)	
CYP2A13	IHC (100% of adenocarcinoma) (18) IHC (93% of squamous cell carcinoma) (18)	
CYP2B7		RNP (100%) (11)
CYP2E1	IHC (46%) (27) IHC (40% of adenocarcinoma) (7)	
CYP3A	IHC (25%) (22) IHC (100%) (26) IHC (40% of adenocarcinoma) (7)	
CYP3A4	TM (4)	
CYP3A5	TM (4)	RT-PCR (50%) (26)
CYP3A7		RT-PCR (13%) (26)
CYP4B1		RNP (100%) (11)
CYP19	IHC (86%) (36)	
CYP24		RT-PCR (56%) (44)
CYP24A1		RT-PCR (43)

Superscript reveals the number of reference. Parentheses indicate % positive rate in tumor tissue or positive level WB: Western blot analysis, IHC: Immunohistochemistry, TM: Tissue microarray , EAA: Enzyme activity assay, RT-PCR: Reverse transcriptase-polymerase, chain reaction, RTQPCR: Real-time quantitative polymerase chain reaction, NB: Northern blot analysis, RNP: RNase protection

characteristic absorption peak of the reduced CO-complex occurs at 450 nm. The CYP family is also a large group of enzymes that catalyze the mono-oxygenation reaction using molecular oxygen and equivalent electrons from NADPH *via* NADPH-dependent P450 reductase. The CYP system plays important roles in the metabolism and excretion of endogenous and exogenous compounds, including different carcinogens such as polycyclic aromatic hydrocarbons, heterocyclic amines, nitrosamines, azo dyes, and alkylating agents (5, 6). Metabolic intermediates of the compounds produced by CYP1A1, CYP2A6, CYP2E1, and CYP3A4/5 in this pathway are often highly active, and are associated with the initiation and promotion of tumor development and progression (5-7).

Many P450 substrates are carcinogens, while other substrates are anticancer drugs. CYPs, therefore, have potentially important roles in tumor biology (8). Although the liver is the major organ to express CYPs that metabolize exogenous chemicals, recent developments in quantitative and qualitative methods for mRNA and protein analyses have shown that many organs and tissues, as well as different types of tumors, express several CYPs (9). Increased CYP expression is frequently observed in tumors, and is important not only for understanding tumor development and progression, but for efficient management of lung cancer also. In the metabolism of anticancer drugs, CYPs are prominent players that enhance or diminish the anticancer function of therapeutic agents. The presence of individual CYPs in lung tumors has been investigated to better understand the intra-tumor metabolism of anticancer agents (9, 10); the results strongly suggest that CYP expression in lung cancer is associated with the prognosis of patients.

Therefore, investigations of tumor-specific CYP expression will provide a basis for the development of novel diagnostic and therapeutic strategies (8). In addition to the metabolism of carcinogens and anticancer agents, CYP19 catalysis of estrogen biosynthesis and CYP24 involvement in vitamin D3 metabolism have been detected in lung cancer tissues, suggesting that CYP19 and CYP24 could be new therapeutic targets for the management of lung cancer.

3. CYP EXPRESSION IN NON-SMALL CELL LUNG CANCER

Cytochrome P450 (CYP) enzymes expressed in human lungs can metabolize a variety of xenobiotics, drugs, and endogenous compounds (11). Metabolism of these substrates leads to their detoxification and/or activation, which may affect the homeostasis of the lung as well as its susceptibility to disease, response to therapy, and clinical prognosis (11). To better understand the importance of drug-metabolizing enzymes in carcinogenesis and the anticancer drug sensitivity of human NSCLC, it is necessary to study the major drug-metabolizing enzyme systems in lung tumors (12).

CYP expression has been studied in human NSCLC as well as in normal tissues. These studies used various methods such as enzyme activity assay (EAA), detection of proteins by immunohistochemistry (IHC) and western blot analysis (WB), tissue microarray (TM), and detection of mRNA by northern blotting (NB), reverse-transcription polymerase chain reaction (RT-PCR), real-time quantitative PCR (RTQPCR), and RNase protection assay (RNP). The results of CYP expression studies in NSCLC as reported by various groups are summarized in Table 1.

The expression of CYP1A1 and CYP1B1 in lungs is transcriptionally up-regulated by the activation of the aryl hydrocarbon receptor (AhR) through binding of ligands such as cigarette smoke components (13). CYP1A1 is the most intensively studied CYP enzyme in the human lung because CYP1A1 is the major enzyme metabolizing polycyclic aromatic hydrocarbon (PAH) and may play an important role in the development of lung cancer through the activation of pro-carcinogens. CYP1A1 mRNA was found in 23% (10/43) cases of lung cancer by NB (14) and in 16% (2/10) cases of lung cancer by RTQPCR (15).

Table 2. Relationship between anticancer drug and CYP metabolism

Anticancer drug		CYP metabolism	
Generic name	Trade name	Activation	Inactivation
Alkaloids			
Irinotecan	Campto, Topotecin		CYP3A4
Paclitaxel	Taxol		CYP2C8, CYP3A4
Docetaxel	Taxotere		CYP3A4
Vinca alkaloids			
Vincristin	Oncovin		CYP3A4
Vinorelbine	Navelbine		CYP3A4
Antimetabolites			
Gemcitabine	Gemzar	-	-
Pemetrexed	Alimta	-	-
Tegafur-Uracil	UFT	CYP2A6, CYP1A2, CYP2E1	
Tegafur-Gimeracil-Oteracil	TS1	CYP2A6, CYP1A2, CYP2E1	
Alkylating agents			
Cyclophosphamid	Endoxan	CYP2B6, CYP2C8, CYP2C9,CYP3A4, CYP2A6	
Molecular targeting agents			
Gefitinib	Iressa		CYP2D6, CYP3A4
Erlotinib	Tarceva		CYP3A4, CYP1A2
Crizotinib	-		CYP3A4 (susp)*
Bevacizumab	Avastin	-	-

These data are extracted from the attachments of anticancer drugs provided by manufacturers

CYP1A1 expression was found in 20% cases of lung cancer (n = 10) by WB (15) and in 44% cases of adenocarcinoma (n = 48) and 37% cases of adenocarcinoma (n = 107) by IHC (7, 13).

CYP1B1 metabolizes the carcinogens associated with tobacco use. This enzyme plays a major role in converting estradiol to its 4-hydroxyl derivative, which is a putative carcinogenic metabolite of estrogen. Using WB and RTQPCR, Spivack *et al.* (15, 16) reported that CYP1B1 was commonly expressed in approximately 100% cases of lung cancer. CYP1B1 expression was found in 47% cases of NSCLC (n = 89) (17) and in 49% cases of adenocarcinoma (n = 107) by IHC (13). Because of its common expression in human lung, CYP1B1 is hypothesized to be an important phase I enzyme with respect to carcinogen metabolism in the human lung (16).

CYP2A13, which is preferentially expressed in the respiratory tract, is the most efficient enzyme for the metabolic activation of tobacco-specific nitrosamines, such as 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK). The relevance of CYP2A13 in carcinogenicity and toxicity in the respiratory tract has been suggested (18). CYP2A13 expression was found in 100% cases of adenocarcinoma (n = 15) and in 93% cases of squamous cell carcinoma (n = 15) by IHC (18). A variant enzyme produced by the genetic polymorphism *CYP2A13*2* was found to be associated with substantially reduced risk for smoking-related lung adenocarcinoma (19), also suggesting the importance of CYP2A13 in the development of lung adenocarcinoma.

CYP2E1 metabolizes some tobacco-specific nitrosamines (20). Carcinogens activated by CYP2E1 are associated with the formation of reactive oxygen species that cause tissue injury (21). CYP2E1 expression was found in 46% of 28 cases of lung cancer by IHC (22) and in 40% of 48 cases of adenocarcinoma by IHC (7). Based on the results from RT-PCR, Raunio *et al.* (23) demonstrated that normal lung tissues also express CYP2E1 mRNA. CYP2A6 expression was found in 46% cases of adenocarcinoma (n = 48) by IHC (7). CYP2B7 and CYP4B1 mRNA were also present in the normal lung and cancer tissues analyzed by RNase protection assay (11). In addition, normal lung tissues were also found to express CYP1A1, CYP3A5, CYP2B7, CYP4B1, and CYP2F1 mRNA, as determined by RT-PCR (23).

Recently, CYPs have been shown to metabolize several essential anticancer agents such as alkaloids, vinca alkaloids, antimetabolites, alkylating agents, as well as the agents used for molecular targeting therapy. Table 2 shows the relationship between anticancer drugs and CYP metabolism. The information was obtained from the manufacturers' documents attached with the anticancer drugs. For instance, CYP3A enzymes not only inactivate the major anticancer drugs, namely, alkaloids and vinca alkaloids, as well as molecular targeting agents, but also activate some anticancer prodrugs such as cyclophosphamide and ifosfamide (24, 25).

Since CYP3A consists of four subfamily members, namely, CYP3A4, CYP3A5, CYP3A7, and CYP3A43, and since the antibodies available from commercial sources usually cross-react with all CYP3A member proteins, the proteins detected with antibodies are described as CYP3A. CYP3A expression was demonstrated in both tumor and normal tissues by IHC (26). CYP3A expression was found in 40% of 48 cases of adenocarcinoma by IHC (7). CYP3A4 has been shown to catalyze the activation of the prodrug ifosfamide, raising the possibility that ifosfamide could be activated in tumor tissues expressing this enzyme (25). CYP3A5 catalyzes the activation of cyclophosphamide and ifosfamide. CYP3A5 mRNA was found in all of eight lung cancers, and CYP3A4 mRNA, in one of eight lung cancers (26). Eight of the 32 (25%) cases of lung cancer showed the expression of CYP3A by IHC (27). Both CYP3A4 and CYP3A5 mRNA (28) and proteins (23, 29) were also expressed in normal lung tissues. Local activation of carcinogens by CYP3A may occur in pulmonary carcinomas and surrounding normal tissues (30). Cyclophosphamide is also activated by CYP2B6 (Table 2). Utilizing an adenoviral expression system, Tychopoulos *et al.* (31) investigated the effects of overexpression of a CYP2B6 and P450-reductase fusion protein on the toxicity of cyclophosphamide in several pulmonary tumor cell lines. Cyclophosphamide toxicity was considerably enhancedby the expression of the

CYP2B6-reductase fusion protein, clearly indicating that CYP2B6 can activate cyclophosphamide.

4. EXPRESSION OF CYP19 (AROMATASE) IN LUNG CANCER

The number of deaths in the female population with pulmonary adenocarcinoma is increasing (32), and it is possibly associated with estrogen function in the lung (30). Estrogen is involved in the differentiation and maturation of normal lungs (33), while it also stimulates the growth and progression of lung tumors (34) through the action of the estrogen receptor (ER) (35). This steroidal growth-stimulatory pathway in tumors may be promoted by the expression and activity of aromatase (CYP19), an estrogen synthase P450 (36). Aromatase (CYP19) synthesizes the estrogen in the adrenals and gonads as well as in the extragonadal tissues, including the brain, skin, and adipose, pancreatic, and lung tissues (36-39). The gene expression of aromatase is regulated by a number of tissue-specific promoters located in the 93-kb 5′-flanking region of the aromatase gene. Demura *et al.* (40) reported that normal lung tissues dominantly used the promoter I.4, which is also used in the skin, and adipose and vascular tissues. In tumor tissues, alternative promoters I.1 (placenta-specific), I.3 (adipose tissue- and ovary-specific), and I.7 (aorta-specific) were utilized at a higher level compared to the normal tissues, although the promoter I.4 was still dominantly used (40). Using immunohistochemical staining, Weinberg *et al.* investigated the expression of CYP19 in lung cancer (n = 53) and reported that CYP19 was detected in 86% of NSCLC, and that the CYP19 enzyme expressed in the tumors was biologically active (Table 1) (36). Utilizing a human lung cancer xenograft model system, Mah *et al.* reported a stimulatory effect of aromatase and estrogen on tumor growth (41). Márquez-Garbán *et al.* (42) also treated lung NSCLC xenografts *in vivo* with an aromatase inhibitor alone and a combination of cisplatin/aromatase inhibitors, and showed a considerable reduction in tumor progression compared to the paired control. Therefore, therapeutic targeting of NSCLC to block the estrogen-signaling pathway may provide new options for the treatment of NSCLC patients (36).

5. INVOLVEMENT OF VITAMIN D3 AND CYP24A1 IN LUNG CANCER

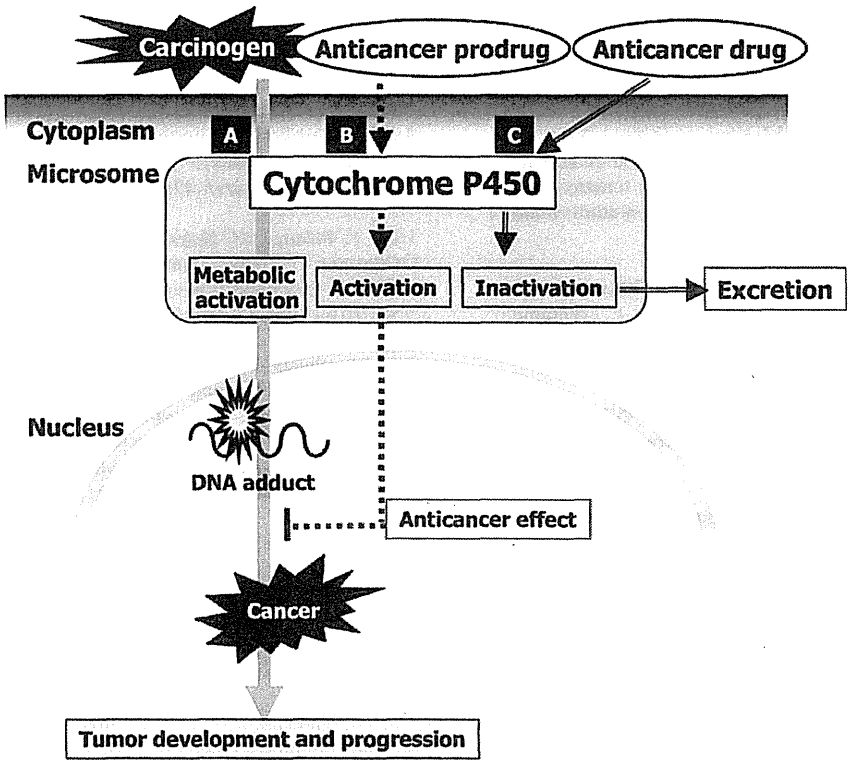
1α,25-Dihydroxyvitamin D3 (1,25-OH D3) and its analogs display potent anti-proliferative activity, mediated by the vitamin D receptor (VDR), in a variety of tumors, and are currently under investigation in clinical trials for anticancer agents (43, 44). Vitamin D3, synthesized by the exposure of skin to light, is converted to 25-hydroxyvitamin D3 by CYP27A1 (vitamin D3 25-hydroxylase P450) in the liver, and further to 1α,25-dihydroxyvitamn D3, the active form of vitamin D3, by CYP27B1 (25-hydroxyvitamin D3 1α-hydroxylase P450) in the kidney (45-48). In 1,25-OH D3-target tissues such as the kidney, small intestine, and bones, CYP24A1 (1,25-hydroxyvitamin D3 24-hydroxylase) converts 1,25-OH D3 to its inactive 24-hydroxyl derivatives (49, 50). The biosynthesis and inactivation pathways of 1,25-OH D3 are important in bone formation and maintenance, and thus, these pathways have been intensively investigated as putative therapeutic targets for the treatment of osteoporosis. In addition to its involvement in bone formation and maintenance, the metabolism of vitamin D3 is also associated with tumor proliferation. The inactivation of 1,25-OH D3 by CYP24A1 expressed in tumor tissues is associated with poor prognosis of some human cancers (43). Upon RT-PCR, CYP24A1 expression was detected in 10 of 18 cases of lung cancer (44), and up-regulated CYP24A1 mRNA expression was also reported in lung cancers (Table 1) (43). The increased CYP24A1 expression observed in lung tumors should restrict 1,25-OH D3 activity (44), suggesting that CYP24A1 could be an alternative target gene for the management of lung cancer.

6. PERSPECTIVES

Since various tumors express a variety of CYP enzymes, CYPs may be good markers for the determination of the quality of lung cancer (7). Drug-metabolizing CYP enzymes could reflect the differences occurring after malignant transformation, and may play a role in the sensitivity of tumor tissues to anticancer drugs. Therefore, we endeavor to discover potential drugs that are specifically activated by the CYPs constitutively expressed in tumors. As summarized in Figure 1, three CYP metabolic pathways can be defined according to the substrate, such as carcinogens, anticancer prodrugs, and anticancer drugs. As shown in Figure 1A, tobacco smoke contains many carcinogens and pro-carcinogens such as benzo(a)pyrene and nitrosamine. Carcinogens such as benzo(a)pyrene are metabolized by phase I enzymes, including CYP family enzymes, and converted to inactive metabolites by phase II enzymes. Benzo(a)pyrene itself, for instance, does not exhibit carcinogenicity, but undergoes metabolic activation by the phase I enzyme CYP1A1 into the diol epoxide, which is mostly converted to an inactive metabolite by the phase II enzyme glutathione S-transferase (GST), particularly the Mu class of GST (GSTM1) (51). However, this epoxide is extremely reactive, and can pave its way into the nucleus to form DNA adducts, which exhibit strong carcinogenicity. CYP-mediated benzo(a)pyrene DNA adducts can cause specific mutations in the p53 gene (52). In Figure 1B, some anticancer drugs are inactivated by CYP. In Figure 1C, some anticancer prodrugs are activated by CYP.

Since substrates such as carcinogens, anticancer prodrugs, and anticancer drugs are activated and/or inactivated by CYP enzymes, in depth study of the local expression of CYP enzymes and their involvement in the metabolism of carcinogens will help explain the mechanisms of carcinogenesis. Based on CYP expression and metabolism by CYP enzymes in lung tumors, new molecular targeting therapy could also be developed with novel agents that are specifically activated in individual lung cancers. As described in this review, aromatase- and CYP24A1-specific inhibitors could also provide novel treatments for non-small cell lung cancer. Therefore, the spectrum of CYP expression in lung tumors will provide

CYP in NSCLC related to exogenous chemical metabolisms



A Carcinogens undergo metabolic activation by CYP, form DNA adducts and then cause cancer.
B Application of a specific CYP expression in tumors as a tumor marker
C Application of CYP-dependent metabolisms for molecular targeting therapy
D Utilities of the knowledge in specific metabolisms by CYPs expressed in tumors for selection of anti-cancer drugs

Figure 1. Cytochrome P450 (CYP) expressed in non-small cell lung cancer (NSCLC). CYP enzymes expressed in NSCLC play roles in the activation of carcinogens and pro-anticancer drugs as well as inactivation of carcinogenic agents and anticancer drugs. Information of CYP expression spectrum in individual tumors may be useful and essential in clinical applications for the better management of lung cancer. *: Crizotinib suspected to be inactivated by CYP3A4.

information for the use of therapeutic agents in a "tailor-made" fashion, whereby we can choose an agent that is efficiently activated, but not easily inactivated, for each individual patient. The spectrum of CYP expression in lung tumors may provide useful tumor markers for the classification and diagnosis of tumors relevant to the management of lung cancer. Thus, studying the local expression of CYP in tumor tissues will provide insights into the mechanisms of carcinogenesis and the intratumoral metabolism of anticancer drugs, which may enable us to developthe present "order-made" therapy to "tailor-made" therapy.

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Abbreviations: CYP: cytochrome P450, SCLC: small cell lung cancer, NSCLC: non-small cell lung cancer, NADPH: nicotinamide adenine dinucleotide phosphate, EAA: enzyme activity assay, IHC: immunohistochemistry, WB: Western blot analysis, TM: tissue microarray, NB: Northern blotting, RT-PCR: reverse transcriptional polymerase chain reaction, RTQPCR: real-time quantitative PCR, RNP: RNase protection assay, AhR: aryl hydrocarbon receptor, PAH: polycyclic aromatic hydrocarbon, ER: estrogen receptor, VDR: vitamin D receptor

Key Words: cytochrome P450, non-small cell lung cancer, NSCLC, anticancer drug, CYP19, CYP24

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Photodynamic therapy as salvage treatment for local failure after chemoradiotherapy in patients with esophageal squamous cell carcinoma: A phase II study

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Local failure at the primary site is a major problem after chemoradiotherapy (CRT) in patients with esophageal squamous cell carcinoma (ESCC). Salvage surgery is the only treatment option with curative intent, but it is associated with high morbidity and mortality. The aim of this study was to evaluate the efficacy and safety of salvage photodynamic therapy (PDT) after CRT. Patients with histologically proven local failure limited to the submucosal layer, and without any metastasis after definitive CRT (≥50 Gy) for ESCC were enrolled in the study. PDT began with intravenous administration of 2 mg/kg of porfimer sodium followed 48–72 hr later by excimer dye laser irradiation with a fluence of 75 J/cm^2. The primary endpoint was a complete response (CR) to treatment with PDT, and the secondary endpoints were toxicity related to PDT, progression-free survival (PFS) and overall survival (OS). Twenty-five patients were enrolled in the study. A CR was attained in 19 of 25 patients treated with PDT (CR rate, 76%; 95% CI, 55–91%). One treatment-related death (4%) caused by gastrointestinal hemorrhage at the irradiated site occurred 33 days after PDT. No adverse events greater than grade 3 were related to PDT in the other patients. After a median follow-up of 48 months after PDT, the PFS and OS at 3 years were 40% (95% CI, 21–59%) and 38% (95% CI, 17–60%), respectively. PDT is a potentially curative and tolerable salvage treatment after CRT for carefully selected patients with local failure without any metastasis.

Chemoradiotherapy (CRT) is a curative treatment option for esophageal squamous cell carcinoma (ESCC). However, local failure without distant metastasis after completion of CRT remains a major problem that must be overcome to achieve a cure. Although salvage esophagectomy is now indicated for such patients, it has a higher morbidity and mortality compared with primary or planned esophagectomy.[1–4] The development of curative and safe salvage treatment options for local failure is needed to improve the survival of patients treated with CRT.

Key words: esophageal squamous cell carcinoma, chemoradiotherapy, photodynamic therapy, salvage treatment

Abbreviations: CR: complete response; CRT: chemoradiotherapy; EMR: endoscopic mucosal resection; ESCC: esophageal squamous cell carcinoma; NCI-CTCAE: National Cancer Institute Common Terminology Criteria for Adverse Events; NSAIDs: non-steroidal anti-inflammatory drugs; OS: overall survival; PDT: photodynamic therapy; PFS: progression-free survival; UMIN: University hospital Medical Information Network

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After completion of CRT, a subset of ESCC patients develops local failure at the primary site without distant metastasis. In such patients, salvage surgery could be a curative treatment option, especially for those with T2 or earlier T-stage tumors or for those without lymph node metastasis.[1,2] Onozawa *et al.* reported that regional nodal failure within the field of elective lymph node irradiation is rare in patients achieving a complete response (CR) after CRT (1%; 95% CI, 0.0–5.3%).[5] These data have encouraged the use of local salvage treatment at only the primary site as a minimally invasive treatment in carefully selected patients.

We reported previously on the potentially acceptable results of endoscopic mucosal resection (EMR) or photodynamic therapy (PDT) as a salvage treatment for local failure after CRT.[6–8] PDT is a more deeply penetrating method than EMR for esophageal cancer even in the salvage setting, because, in our experience, PDT can cure patients with deep invasion of the submucosal layer or T2 local failure. In addition, PDT can be indicated both as a curative treatment for superficial esophageal cancer[9,10] and as a palliative treatment to relieve dysphagia caused by stenosis in more advanced esophageal cancer.[11] We believe that PDT might be a curative and effective treatment option for patients with local failure at the primary site after definitive CRT. We conducted a prospective study to evaluate the efficacy and safety of salvage PDT after CRT.

Material and Methods

This was a single-arm, open-label, single-center phase II study. The primary endpoint of this study was the CR rate at the primary site after PDT. The secondary endpoints were toxicity related to salvage PDT, progression-free survival (PFS) and overall survival (OS). All adverse events were evaluated according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE) version 3.0.[12] The study protocol was approved by the institutional review board of the Japanese National Cancer Center in January 2005. The study was carried out according to the ethical principles of the Declaration of Helsinki. Before enrollment, all patients provided written informed consent. This study was registered with the University hospital Medical Information Network (UMIN) Clinical Trials Registry, and the identification number is C000000244.

Eligibility and exclusion criteria

The eligibility criteria of this study were as follows: (*i*) local failure after definitive CRT (≥50 Gy) for ESCC; (*ii*) the patient's refusal to undergo salvage surgery; (*iii*) histologically proven squamous cell carcinoma by biopsy specimen of the local failed lesions; (*iv*) local failed lesions limited to the submucosal layer; (*v*) EMR not indicated for reasons of concomitant deep ulceration, severe fibrosis caused by radiation or a lesion invading to the deep submucosal layer; (*vi*) Eastern Cooperative Oncology Group performance status ≤2; (*vii*) adequate bone marrow function (white blood cell count ≥2,000/mm,^{3} platelet count ≥75,000/mm^3), renal function (serum creatinine level ≤2.0 mg/dL) and liver function (serum bilirubin level <2.0 mg/dL, both alanine aminotransferase and aspartate aminotransferase <100 IU/L) and (*viii*) provision of written informed consent. The exclusion criteria were as follows: (*i*) active malignancy other than early gastrointestinal cancer that was curable with endoscopic treatment within 1 year; (*ii*) systemic infection requiring antibiotics; (*iii*) significant cardiovascular disease (uncontrolled hypertension, myocardial infarction, unstable angina, congestive heart failure), uncontrolled diabetes mellitus, or liver cirrhosis; (*vi*) baseline stage T4 before CRT; (*v*) presence of lymph node or distant metastasis confirmed by computed tomography (CT) after CRT and (*vi*) known porphyria.

Evaluation of baseline clinical stage and the effect of CRT

Baseline clinical stage was determined using the TNM classification of the International Union Against Cancer.[13] Clinical T stage was evaluated by endoscopy, endoscopic ultrasound (EUS) and CT of the chest. Clinical N and M stages were evaluated by EUS and CT of the neck, chest and abdomen. In this study, lymph node metastasis was diagnosed clinically if the lymph node was ≥10 mm in diameter on CT. After completion of CRT, all patients were followed-up with both endoscopy and CT at 1, 3, 6, 9 and 12 months, and then every 4 months after completing CRT.

Evaluation of the local failure at the primary site after CRT

Before PDT, the depth of all failure lesions was evaluated using EUS (EU-M2000, Olympus Co. Ltd., Tokyo, Japan). We carefully observed the lesions with a high-frequency (20 MHz) miniature probe. When we detected a hetero-echoic solid component in the submucosal layer, we diagnosed it as a local failure lesion.

PDT treatment and surveillance

All PDTs were performed as inpatient procedures. PDT began with intravenous administration of 2 mg/kg of porfimer sodium (Photofrin, Pfizer Japan Inc.) followed by excimer dye laser irradiation. Porfimer sodium was reconstituted as a 2.5 mg/mL solution in 5% glucose. It was injected within 5 min, and the injection rate was less than 12 mL/min. A 630 nm wavelength laser beam was emitted by an excimer dye laser (EDL-1, Hamamatsu Photonics, Hamamatsu, Japan), and the laser light was delivered *via* a microlens- tip fiber, without any balloon or light diffuser, through the operative channel of the scope. An attachment was fitted to the tip of the scope to keep it facing the lesion and to maintain the distance between the tip of microlens fiber and the surface of the lesion during the procedure. The laser treatment was performed 48 hr after the injection of porfimer sodium. The fluence was 75 J/cm^2, with a fluence rate of 160 mW/cm^2 (4 mJ/pulse, 40 Hz pulse frequency). If the lesions were larger than 1 cm^2, multiple treatment fields were overlapped to cover the entire lesion. If the effect (*e.g.*, ischemic change of mucosa) after the laser treatment change, as evaluated by endoscopic observation was insufficient, additional laser irradiation was performed at a second session, 72 hr after the injection.[8,14,15]

All patients were instructed to avoid direct exposure to sunlight for 1 month after the injection of porfimer sodium to protect them from the adverse effects of skin photosensitization. Patients were discharged 2 weeks after laser irradiation, if there were no complications related to PDT. Adverse events were identified through a physical examination and endoscopic evaluation performed every 2 weeks until 2 months after PDT. One month after PDT, patients were assessed through a physical examination, measurement of haematological and biochemical variables in blood and endoscopic examination. The endoscopic examination with biopsy was repeated at least every month thereafter to evaluate the response and luminal toxicity of PDT until the response was confirmed. CT was used to evaluate distant organ or lymph node metastasis every 3 months for the first 2 years and every 6 months thereafter.

Statistical analysis

The primary endpoint of this study was the CR rate with salvage PDT. The sample size was determined assuming a binomial distribution. A threshold CR rate was considered to be 30%, and a CR rate of 60% was considered to be of potential interest. The planned accrual was calculated as 25 patients

Table 1. Baseline patients' characteristics before CRT ($n = 25$)

Characteristics	Number of patients
Sex	
Male	23
Female	2
Median age	67 years
(range)	55–82
Location	
Upper	4
Middle	19
Lower	2
Histology	
W/D,SCC	0
M/D,SCC	7
P/D,SCC	3
SCC	15
Baseline TNM stage	
Stage I	5
Stage II	11
Stage III	7
Stage IVA	2
T stage	
T1	6
T2	7
T3	12
N stage	
N0	16
N1	9

Abbreviations: W/D, well differentiated; SCC, squamous cell carcinoma; M/D, moderate differentiated; P/D, poorly differentiated.

Table 2. Patients' characteristics before PDT ($n = 25$)

Characteristics	Number of patients
Regimen of chemotherapy	
Cisplatin + 5FU	23
Others	2
Radiation dose (Gy)	
50.4	15
≥60	10
Local failure pattern after CRT	
Recurrent	14
Residual	11
Lesion circumference of the lumen	
<1/4	10
1/4–1/2	15
Concomitant ulceration on the lesion	
Present	6
Absent	19

Abbreviation: 5FU, 5-fluorouracil.

(allowing for 10% ineligibility) with $\alpha = 0.1$ and $\beta = 0.1$. If the calculated one-sided lower 95% confidence limit of the CR rate was $\geq$ 30%, the primary endpoint was considered to have been met. The PFS was measured from the date of enrollment to the first date of recurrence, disease progression at any site, or death. The OS was measured from the date of enrollment to the date of death for any reason or to the last follow-up visit. Survival time was calculated by the Kaplan-Meier method. Survival time was compared between variables by using the log-rank test. An alpha value of < 0.05 was considered significant. All statistical analyses were performed using Predictive Analysis Software Statistics 18 (SPSS Japan Inc., Tokyo, Japan).

Results

Between April 2005 and January 2009, a total of 34 patients were recruited for this study. Nine of these patients were deemed ineligible (one with an active other malignancy within 1 year, seven with baseline stage T4 before CRT and one with a distant metastasis); thus, 25 patients were enrolled in this study. All 25 patients were treated with salvage PDT. The patients' baseline characteristics before CRT are summarized in Table 1. The patients included 23 men and two women, and the median age was 67 years (range, 55–82 years). The tumor location was the upper esophagus in four patients, middle esophagus in 19 patients and lower esophagus in two patients. The baseline clinical stages before CRT were: stage I in five, stage II in 11, stage III in seven and stage IVA in two patients, and no patient had distant organ metastasis before CRT. The patients' characteristics before PDT are summarized in Table 2. Most of the chemotherapeutic regimens of CRT comprised cisplatin and 5-fluorouracil with ≥50 Gy concomitant radiotherapy. Their failure patterns were recurrence after achieving a CR with CRT in 14 patients and residual lesions after CRT in 11 patients. All local failure lesions in this study were histologically proven T1b lesions within the radiation field. The median duration between the last day of radiation and the initiation of PDT was 192 days (range, 21–1,234 days).

Efficacy

In this study, the range of esophageal surface areas that were treated was 3–9 cm^2.CR was attained in 19 of 25 patients with PDT, resulting in a CR rate of 76% (95% CI, 55–91%). A representative case of a patient who achieved CR is shown in Figure 1. There was no dose-response relationship in this study. The median esophageal surface area was 6 cm^2 in 19 patients who achieved CR and in six patients who did not achieve CR with PDT. The relationship between the degree of baseline lymph node metastasis and CR rate was as

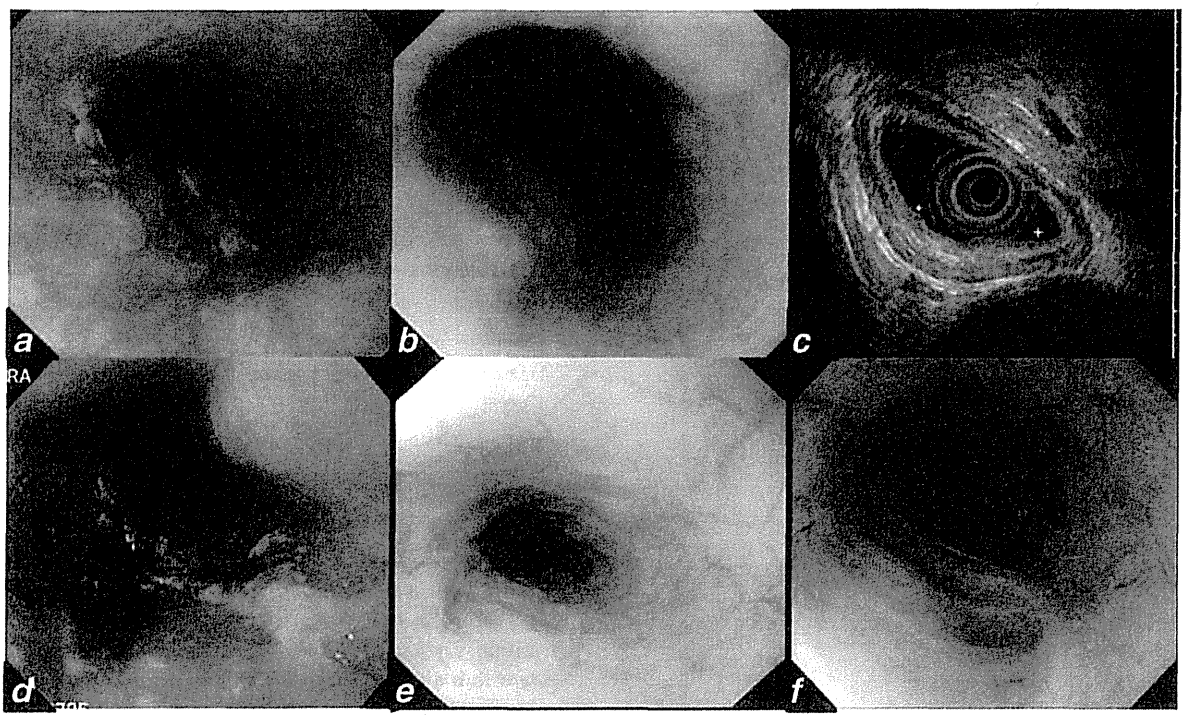

Figure 1. A patient who achieved a complete response (CR) with salvage photodynamic therapy (PDT) is presented. (*a*) Before chemoradiotherapy (CRT), the baseline stage was T2N0M0. (*b*) A local residual lesion was detected at the primary site after CRT. (*c*) The residual lesion was limited to the submucosal layer. (*d*) Two days after PDT, an ischemic change was observed at the laser-irradiated site. (*e*) One month after PDT, deep ulceration was observed at the laser-irradiated site. (*f*) A CR was achieved, and there was no recurrence at the primary site 3 years after PDT.

Table 3. Adverse events after PDT ($n = 25$)

	Grade (no. of patients)					
Adverse events	**1**	**2**	**3**	**4**	**5**	**% (any)**
Pain-Pharynx	3	1	0	0	0	17
Pain-Chest	11	3	0	0	0	61
Anorexia	1	0	0	0	0	4
Dysphagia	7	2	0	0	0	39
Nausea	1	0	0	0	0	4
Vomiting	1	0	0	0	0	4
Fever	11	0	0	0	0	48
Photosensitivity	7	1	0	0	0	32
Hemorrhage-GI	0	0	0	0	1	4

Abbreviation: GI, gastrointestinal.

follows: the CR rate of 16 N0 patients was 75% (12/16), whereas the CR rate of 9 N1 patients was 78% (7/9). The relationship between the baseline T stage before CRT and CR rate was as follows: the CR rate with baseline T1 or T2 was 85% (11/13, 95% CI, 55–98%), whereas that with baseline T3 before CRT was 67% (8/12, [95% CI, 35–90%]). Furthermore, the 1-year local control rate of patients with baseline T1 or T2 was significantly higher compared with that of patients with baseline T3 (T1 or 2 *vs.* T3 = 77% [95% CI, 54–100%] *vs.* 42% [95% CI, 14–70%], $p = 0.04$).

Safety

The safety of PDT in all 25 patients is shown in Table 3. Common adverse events after PDT were chest pain (61%), pharyngeal pain (17%), dysphagia (39%) and fever (48%). Photosensitivity was observed in eight (32%) patients. All patients' fevers were grade 1 with NCI-CTCAE, and most patients recovered within a day. Predose nonsteroidal anti-inflammatory drugs (NSAIDs) might not have been necessary based on the results of this study, because patients' fevers were not severe nor prolonged. Severe complications (≥grade 3) related to PDT limited to one patient death due to gastrointestinal hemorrhage 33 days after PDT. His baseline stage before CRT was T3N0M0, and a histologically confirmed local residual lesion was detected after CRT. After enrollment in this study, he was treated with a fluence of 75 J/cm^2 and a fluence rate of 160 mW/cm^2 for the treatment area of 9 cm^2. He received the maximum treatment field with the largest light dose in this study. He complained of continuous chest pain (grade 2) after PDT, but his pain was controlled with

oral administration of a NSAID. Although we could not confirm the origin of the hemorrhage with endoscopic observation or autopsy, deep ulceration was observed endoscopically at the PDT-irradiated site 1 week before his death. We thought that the hemorrhage was caused by an aortic-esophageal fistula at the laser-irradiated site. The death of this patient gave a 4% (1/25) rate of treatment-related death. No other patient developed an esophageal fistula. Six patients (24%) developed esophageal stenosis requiring balloon dilatation.

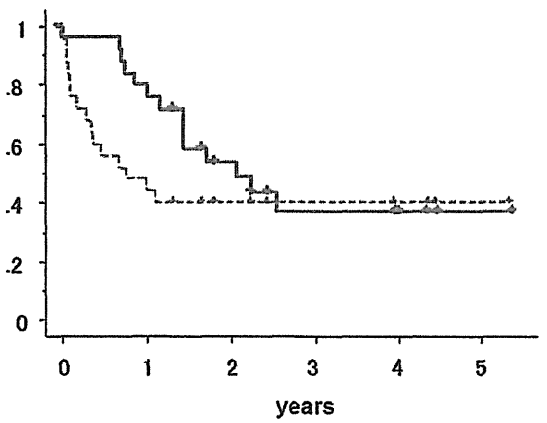

Figure 2. Progression-free survival (red dotted line) and overall survival (blue line) of 25 patients after the initiation of salvage photodynamic therapy (PDT).

Survival

The median follow-up was 48 months (range, 17–64 months). The clinical courses of the 19 patients who had achieved a CR with PDT were as follows. Of the 11 patients who did not develop recurrence, ten are still alive and one died of multiple liver metastases from a prior gastric adenocarcinoma without any esophageal cancer recurrence. Among the remaining eight patients, three developed local recurrence, and all three were treated with salvage esophagectomy, but none survived. Local recurrence was detected within a year (range, 5–10 months) after achieving CR in all three patients, and therefore, the local control rate at 1 year was 64% (16/25, [95% CI, 43–82%]). Lymph node metastasis without local recurrence was detected in three patients; one underwent surgery and the other two were treated with systemic chemotherapy, but all died of cancer progression. Two patients developed liver metastasis and were treated with systemic chemotherapy; one died because of disease progression,

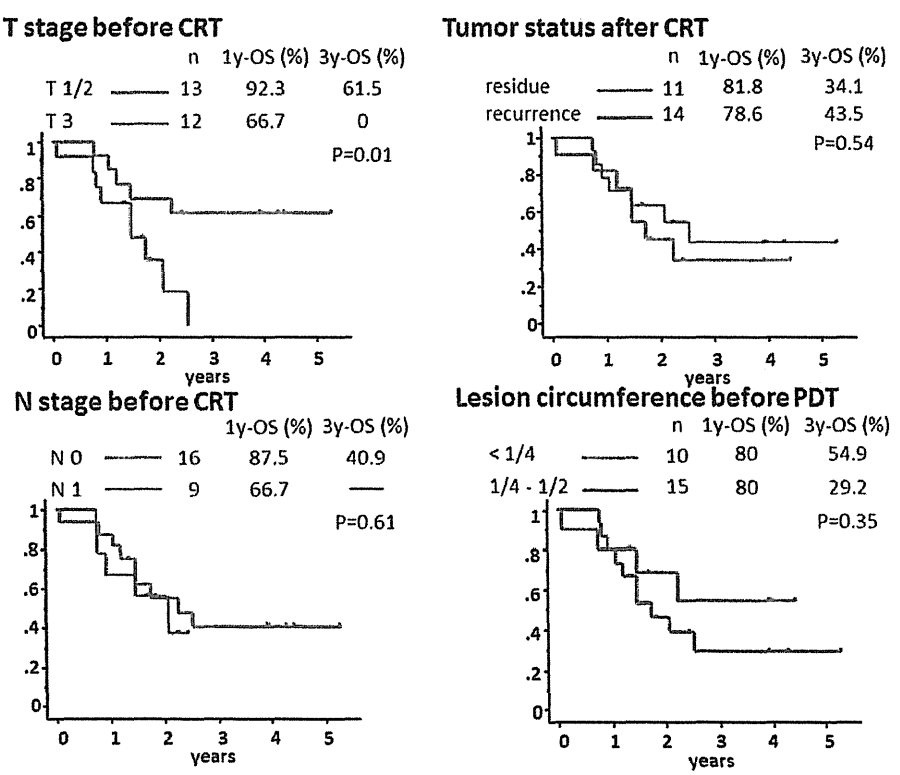

Figure 3. Comparisons of overall survival according to various clinical variables before chemoradiotherapy and before photodynamic therapy.

and the other is still alive about 2 years after detection of liver metastasis. Six patients could not achieve a CR with PDT. Two were treated with systemic chemotherapy, two received salvage surgery and one was treated with a second PDT; all died because of disease progression. The remaining patient's death was classified as a treatment-related death, as described earlier. The PFS rates of all 25 patients at 1 and 3 years were 48% (95% CI, 28–68%) and 40% (95% CI, 21–59%), respectively, and the OS rates at 1 and 3 years were 80% (95% CI, 64–96%) and 38.4% (95% CI, 17–60%), respectively (Fig. 2). Comparisons of OS according to various clinical variables before CRT and before PDT are presented in Figure 3. Patients with clinical T1 or T2 before CRT had significantly higher OS than those with clinical T3 before CRT (T1 or T2 *vs.* T3: 1-year OS = 92.3% [95% CI, 77.8–106.8%] *vs.* 66.7% [95% CI, 40–93.3%], 3-year OS = 61.5% [35.1–88%] *vs.* 0%, $p = 0.01$), whereas there was no significant difference between patients with clinical N0 and N1 before CRT (N0 *vs.* N1: 1-year OS = 87.5% [95% CI, 71.3–103.7%] *vs.* 66.7% [95% CI, 35.9–97.5%], 3-year OS = 40.9% [95%CI, 16–65.8%] *vs.* not reached, $p = 0.61$). There was no difference in OS between patients with a residual lesion after CRT and a recurrent lesion after achieving CR (residual *vs.* recurrent: 1-year OS = 81.8% [95% CI, 59.0–104.6%] *vs.* 78.6% [95% CI, 57.1–100%], 3-year OS = 34.1% [95% CI, 4.8–63.4%] *vs.* 43.5% [95% CI, 14.4–72.6%], $p = 0.54$). Patients with a local failure lesion less than 1/4 the circumference of the lumen had a better OS than those with 1/4 to 1/2 circumference lesions; however, the difference was not statistically significant (<1/4 *vs.* 1/4-1/2: 1-year OS = 80% [95% CI, 55.2–104.8%] *vs.* 80% [95% CI, 59.8–100%], 3-year OS = 54.9% [95% CI, 21.1–88.7%] *vs.* 29.2 [95% CI, 4.1–54.3%], $p = 0.35$).

Discussion

To our knowledge, this is the first prospective study of salvage treatment for local failure after definitive CRT in patients with ESCC. In this study, the primary endpoint (CR rate) was met, and the results exceeded our expectations. The CR rate at the primary site was 76% (95% CI, 54.9–90.6%), suggesting that salvage PDT could be a curative treatment option for carefully selected patients with local failure at only a primary site after CRT. The 3-year survival rate of salvage PDT was 38.4%. This result indicates that salvage PDT can cure a subset of patients with local failure after CRT.

If the failure lesions are tiny and superficial, EMR could be a salvage treatment option for local failure after CRT. We have reported the long-term results for salvage EMR, and the 5-year survival rate was 49.1%.[7] In our report, more than half of the patients had baseline clinical T1 lesions before CRT, and all their local failure lesions were within the submucosal layer before EMR.[7] By contrast, in this study about half of the patients (12/25) had baseline clinical T3 lesions before CRT. Salvage EMR is technically difficult if the failure lesion is severely fibrotic after CRT or there is deep invasion of the submucosal layer. PDT could be a treatment option if local failure after CRT is limited to the submucosal layer without lymph node metastasis and in patients for whom surgery would be intolerable because of physical limitations. Therefore, PDT has a niche role between EMR and surgery in the salvage setting after CRT.

In general, salvage surgery is indicated for patients with local failure after CRT. However, the most serious problems with salvage surgery are the high rates of complications and treatment-related mortality. Compared with esophagectomy without CRT or esophagectomy after planned neoadjuvant CRT, salvage surgery is associated with several complications, such as a longer hospital stay and higher anastomotic leak rate. The treatment-related mortality rate ranges from 8 to 22%.[1–4,16] Therefore, the indications for salvage surgery should be carefully considered. Although treatment-related death occurred in one patient in this study, the incidence rate (4%) was lower than that for salvage surgery. This suggests that salvage PDT is a less morbid treatment option than salvage surgery for carefully selected patients with local failure at the primary site after CRT.

In this study, five patients received salvage surgery for local failure after PDT. Although their physical condition was evaluated as tolerable for salvage surgery, they refused surgery before enrollment in this study. When the failure after PDT was detected, we informed them that their failure lesions were unlikely to be cured with reapplication of PDT because their lesions were suspected to be progressive refractory tumors; they then accepted salvage surgery. None of these patients achieved cure with salvage esophagectomy after PDT, and their median survival time after esophagectomy was 13 months (range: 4–18 months).

At present, nine patients remain alive without disease and one patient is alive with liver metastasis and is being treated with systemic chemotherapy. All of these patients survived with esophagus preservation. Second-line chemotherapy is one treatment option for patients with residual ESCC after CRT, although it is not curative and has a limited effect; that is, the overall response rate of second-line chemotherapy is low (0–16%), and a CR is difficult to achieve (0–6%).[17–20] This suggests that second-line systemic chemotherapy is a palliative treatment.

From the results of a comparison of OS according to various clinical variables, patients with T1 or T2 stage before CRT had a significantly higher survival rate than those with T3 lesions before CRT. All failure lesions in this study were determined before PDT to be within the submucosal layer; however, more advanced failure lesions might be included in the T3 group because of the difficulty of EUS evaluation after CRT, especially in advanced cases. However, N stage before CRT did not affect the survival after PDT. Patients with earlier T stage before CRT tend to be cured with salvage PDT, and these data demonstrate the reproducibility of our retrospective analysis.[14]

Before this phase II study, we did not perform the laser dose escalation study for local failure after CRT for