Results

Patient characteristics

There were 110 patients who received definitive or palliative CRT during the periods: 110 patients matched the recruitment criteria, and 29 patients were excluded from the analysis. The reasons for exclusion were stage IVB (5 patients), double cancer (3 patients), age > 75 years (10 patients), inadequate organ function (5 patients), fistula (2 patients), small cell carcinoma (1 patient), and carcinoma of the cervical esophagus (3 patients). The characteristics of the participating 81 patients are listed in Table 1. Seventy-four patients were men and 7 were women, and the median age was 63 years. Most patients had a good performance status. According to our criteria, the clinical staging was classified as follows: stage I in 8 patients, stage II in 14 patients, stage III in 25, and stage IV in 34. There were 22 patients (27%) with T3 disease, and 44 (54%) with T4 disease. Of 81 patients, 33 (41%) had M1 LYM disease. Clinically involved sites in the 44 cases with T4 disease were thoracic aorta (28 patients), tracheobronchial tree (13 patients), and both sites (3 patients). Five patients had cervical node metastasis, 25 had abdominal nodes, and 3 had metastases in both nodes. All 81 patients had histopathologically confirmed squamous cell carcinoma. Seventy-five patients (93%) completed at least the CRT segment with a total radiation dose of 60 Gy. The remaining 6 patients did not complete CRT; 4 experienced disease progression, and 2 died of treatment-related esophagoaortic fistula. Of 63 patients who responded to CRT, 55 (87%) received the additional two or more courses of chemotherapy.

Table 1. Patient characteristics

No. of patients	81
Sex (male/female)	74/7
Age, years (range)	63 (45–75)
Performance status	
0/1/2	64/13/4
Location[a]	
Upper	8
Middle	43
Lower	30
Histopathology	
Well differentiated	9
Moderately differentiated	58
Poorly differentiated	14
Stage (UICC)	
Stage I	8
Stage II	14
Stage III	25
Stage IV	34
Tumor[b]	
1	10
2	5
3	22
4	44
Metastasis[b]	
0	48
1a	33

[a] Location of the tumor according to the TNM classification (UICC, International Union Against Cancer)
[b] Numbers correspond to the tumor-node-metastasis system of classification

Clinical response to chemoradiotherapy

Of the 81 eligible patients, 34 (42%) achieved CR. The CR rates in patients with stage I, II, III, and IV were 100% (8 of 8 patients), 57% (8 of 14), 36% (9 of 25), and 26% (9 of 34), respectively. Although the CR rate in patients with T4 tumors was 18% (8 of 44 patients), the CR rate in patients with non-T4 tumors was 70% (26 of 37). A significant difference was seen between T4 and non-T4 ($P < 0.0001$). Furthermore, the CR rate in patients with M1 LYM (8 of 33; 24%) disease was significantly different from that in patients with M0 LYM (26 of 48; 54%, $P = 0.0073$). Of 25 patients with T4M1 disease, 4 (16%) achieved a CR and 2 (8%) had more than 3-year survival.

Survival

After a median follow-up period of 57 months (range, 34–114 months), the median survival time of the 81 patients was 14 months. One- and 3-year overall survival rates were 62% (50 of 81) and 22% (18 of 81), respectively. The median survival times of 37 patients with T1–T3 disease and 44 patients with T4 disease were 35 and 11 months, respectively; the survival rate of patients with T1–T3 disease was significantly longer than that of patients with T4 disease ($P = 0.001$, log-rank test). Figure 2 shows the survival curves of 81 patients based on the CR rates (CR or non-CR). There was a

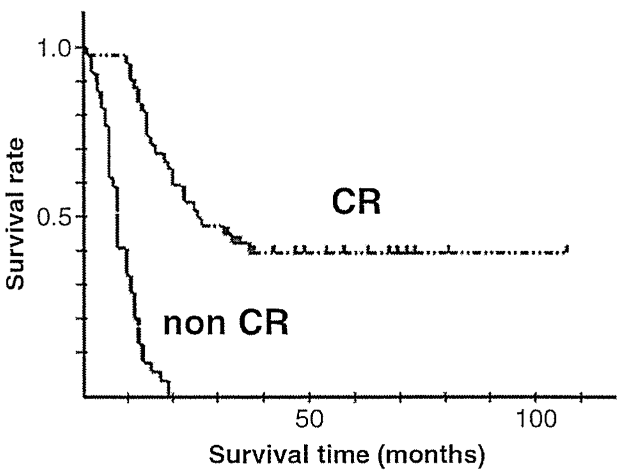

Fig. 2. Survival curves for 34 patients with complete response (*CR*) and for 47 patients without complete response (*non CR*)

Table 2. Major complications appearing during chemoradiotherapy

	Grade 1		Grade 2		Grade 3		Grade 4	
	n	%	*n*	%	*n*	%	*n*	%
Leukocytopenia	15	19	32	40	20	25	4	5
Anemia	4	5	31	39	26	33	0	0
Thrombocytopenia	7	9	9	11	7	9	4	5
Nausea/vomiting	24	30	7	9	13	16	0	0
Diarrhea	7	9	15	19	7	9	3	4
Mucositis	9	11	9	11	7	9	0	0
Esophagitis	32	40	15	19	13	16	7	9
Renal	7	9	15	19	0	0	0	0

Table 3. Patients with grade 2 or greater late cardiopulmonary toxicities

Case No.	Cardiac Ischemia (grade)	Pericardial Effusion (grade)	Heart Failure (grade)	Pleural Effusion (grade)	Radiation Pneumonitis (grade)	D/A	Survival (months)
1	3					Alive	37
2	4					Alive	114
3	4		4			Died	39
4		3		2		Alive	50
5		4	4			Died	14
6		4	4			Died	20
7				2		Alive	67
8		2		3		Alive	72
9		2		3		Alive	37
10		2		3	4	Died	45

D, died; A, alive; NCI-CTC, National Cancer Institute Common Toxicity Criteria; RTOG, Radiation Therapy Oncology Group; EORTC, European Organization for Research and Treatment of Cancer
Source: NCI-CTC version 2.0, RTOG / EORTC late radiation morbidity scoring scheme

significant difference between CR and non-CR cases ($P < 0.0001$). In the CR cases, the patterns of first treatment failure were local recurrence in 3 patients, pleuritis carcinomatosa in 1 patient, liver metastasis in 2 patients, distant lymph node metastasis in 4 patients, bone metastasis in 1 patient, and brain metastasis in 1 patient, respectively. Of 34 CR cases, 12 patients (35%) died of distant or local recurrence of ESCC, while the frequency of local recurrence was low (25%). In contrast, 4 patients died as a result of late toxicities without carcinoma.

Acute toxicity

The major side effects of CRT encountered in our patients during treatment (Table 2) included myelosuppression and esophagitis. Grade 3 and higher leukocytopenia, anaemia, thrombocytopenia, and esophagitis occurred in 30%, 33%, 14%, and 25% of the patients, respectively. Two patients (2%) developed sepsis associated with leukocytopenia; however, these patients recovered from sepsis with use of filgrastim and antibiotics. Of 44 patients with T4 disease, 3 (7%) developed treatment-related perforation of the esophageal wall: 1 developed a mediastinal fistula whereas each of the other 2 developed an aortic fistula. These 3 patients had T4 disease before treatment, and these events occurred during chemoradiation. One patient showed spontaneous healing of the mediastinal fistula after the disappearance of inflammatory findings, despite continuation of treatment, and achieved a PR. The remaining 2 patients with esophagoaortic fistulae died of massive bleeding during two and four courses of chemoradiation, respectively.

Late toxicity

Grade 3 and higher cardiopulmonary toxicities are summarized in Table 3. The median time to the onset of pericarditis from the initiation of treatment was 19 months, ranging from 10 to 36 months for grade 2 and higher pericarditis. Of 3 patients with grade 3 and higher pericarditis, 2 patients suffered grade 4 heart failure at 12 and 20 months after initiation of treatment, respectively, and died as a result of heart failure without cancer recurrence (cases 5 and 6). Two patients with grade 2 pericarditis were manageable with diuretics (cases 8 and 9). Three patients suffered ischemic heart disease at 28, 30, and 54 months, respectively, after the initiation of treatment. One patient suffered grade 3 ischemic change at 28 months, and is alive taking a medicine without cancer recurrence 9 months later (case 1). Another patient suffered grade 4 acute myocardial infarction at 54 months and is alive after receiving percutaneous transluminal coronary angioplasty (PTCA) without cancer recurrence 40 months later (case 2). The third patient suffered grade 4 acute myocardial infarction at 36 months and died as a result of heart failure without cancer recurrence 3 months later (case 3).

The median time to the onset of pleural effusion from the initiation of treatment was 16 months, ranging from 5 to 39 months for grade 2 and higher pleural effusion. Of three patients who required pleurocentesis, all patients suffered grade 2 pericarditis, and one of three patients suffered grade 4 radiation pneumonitis simultaneously at 30 months (case 10). This patient without cancer recurrence died as a result of pneumonia 6 months later, although this patient received corticosteroid therapy for radiation pneumonitis. Two patients with grade 2 pleural effusion were manageable with only diuretics (cases 4 and 7). Two patients suffered benign esophageal stricture and required frequent repeated esophageal dilatation.

In total, four patients died without cancer recurrence, and these causes of death may have been related to cardiopulmonary toxicity.

Discussion

The value of CRT for the treatment of unresectable esophageal carcinoma remains controversial, and only a few clinical studies have been published since the 1980s.[3–8,13] Although some reports included patients with T4 disease, the proportion of such patients was usually low and the clinical outcomes were not clearly described. The results of these studies, including CR rates and survival rates, were confusing, because the clinical and pathological backgrounds varied, especially with regard to the stage of the disease. Therefore, stratification by clinical stage should be applied when evaluating the impact of treatment on survival and response. Zeone et al. reported the results of curative nonsurgical treatment that consisted of 5-FU, CDDP, and 64 Gy radiotherapy combined with neodymium:yttrium-aluminium garnet (Nd:YAG) laser therapy in appropriate patients.[21] They treated 65 patients who had predominantly T1–T3 disease, but their study included 5 patients with T4 disease. Although the 3-year survival rate of the 65 eligible patients was 37%, all 5 patients with T4 disease died within 18 months. However, clinical stages based on the TNM classification were not described; therefore, the stage at which patients survived longer is unknown. Although there are some "gray zones" with respect to determining T3 or T4 disease by imaging, several studies have reported the successful use of CT scans and/or magnetic resonance imaging with an accuracy rate ≥80%.[17,18] We adopted their reported criterion to define T3 or T4 disease. Diagnostic radiologists, together with medical oncologists, were responsible for the final staging. A literature search produced no other studies that specifically investigated CRT for locally advanced disease, such as T4 and/or M1 LYM. In our previous study, a CR rate of 33% and a 3-year survival rate of 23% were achieved in patients with unresectable T4 tumors and/or M1 LYM disease,[15] suggesting that concurrent CRT was potentially curative for locally advanced carcinoma. Although our study, including 40 patients with T4 and/or M1 LYM, was retrospective and may be biased, the results with a 3-year survival of 30% were comparable with the reported trials of CRT, including RTOG studies.[22,23] The pattern of failure in all eligible patients of our study showed that local failure was still dominant, and the RTOG study also showed a similar pattern of failure for CRT. Improvement of local control may affect survival. In contrast, the pattern of failure in CR patients showed that distant metastasis was dominant and local recurrence was not dominant. Furthermore, fatal complication due to radiation-induced late toxicity was found in patients who achieved CR. Therefore, the dose-escalation strategy of radiotherapy was not thought to be effective in patients with ESCC.

Radiation-induced heart disease is one of the complications in patients who undergo thoracic radiotherapy.[24,25] Pericardial disease is the most common manifestation of radiation-induced heart disease. There have been many reports of pericardial disease after thoracic radiotherapy in patients with malignant lymphoma.[26–28] According to recent observation, ischemic heart disease after thoracic radiotherapy is not negligible, and it should be considered in the radiotherapy treatment planning.[29–32] We observed 2 patients with myocardial infarction and 1 patient with angina pectoris, although we were not sure whether these were related to the CRT. Because daily consumption of alcohol and cigarettes are high risk factors for coronary disease, ischemic heart disease may occur in our subjects with esophageal carcinoma. All 3 patients with ischemic heart disease smoked cigarettes daily. The patient with angina pectoris was accompanied by hyperlipemia. One of 2 patients with myocardial infarction had hypertension, hyperlipemia, and diabetes mellitus. However, the possibility that ischemic heart disease is related to late toxicity for CRT is not denied because there have been few reports on pericardial disease in patients with esophageal carcinoma. There have been many reports with regard to definitive CRT for ESCC, and survival time was improved as a result of wide-range radiotherapy combined with use of chemotherapy. In our study, myocardial infarction was found in 2 patients, while 1 patient accompanied by pericardial effusion (grade 3 pericarditis) died and another patient without effusion is still alive more than 3 years after PTCA. Further, 1 patient with heart failure accompanied by pericardial effusion (grade 3 pericarditis) died. Treatment of pericardial effusion should be actively performed, and medication, pericardiocentesis, or pericardial window placement are manageable.[24,33] Radiation-induced late toxicity, such as acute myocardial infarction and pericarditis, can cause the risk of treatment-related death even in CR cases. Examination to evaluate the function of the heart should be performed constantly. In contrast, the incidence of grade 2 and more benign pleural effusion in our study was 15% in 34 CR cases, and we believe that benign pleural effusion is common in patients after definitive CRT. The cause of the pleural effusion may be related to heart disease, such as heart failure and pericardial effusion. Treatment of pleural effusion, such as medication, pleurocentesis, and pleurodesis, should be actively performed.

Severe late toxicities, such as myocardial infarction, pericardial effusion, and pleural effusion, were found in patients who achieved CR after definitive CRT. The definitive CRT is potentially curative and is improved survival time, while treatment-related death of radiation-induced late toxicities was found in 11% of CR cases. More than half of the entire heart volume received at least 40 Gy radiation in most patients. Three-dimensional conformal radiotherapy and intensity-modulated radiotherapy have potential advantages over traditional radiotherapy in reducing doses to the heart. Because a precise analysis using a dose–volume histogram is performed, a decreasing radiation volume to the heart will be required due to reducing radiation-induced late toxicities.

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Study of *p53* gene alteration as a biomarker to evaluate the malignant risk of Lugol-unstained lesion with non-dysplasia in the oesophagus

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Mutations of the *p53* gene are detected frequently in oesophageal dysplasia and cancer. It is unclear whether Lugol-unstained lesions (LULs) with non-dysplastic epithelium (NDE) are precursors of oesophageal squamous cell carcinoma (ESCC). To study the genetic alterations of NDE in the multistep process of oesophageal carcinogenesis, we determined the relationship between *p53* mutations and LULs-NDE. Videoendoscopy with Lugol staining was performed prospectively in 542 oesophageal cancer-free subjects. Lugol-unstained lesions were detected in 103 subjects (19%). A total of 255 samples, including 152 LULs (NDE, 137; dysplasia, 15) and 103 paired samples of normal staining epithelium, were obtained from 103 subjects. After extraction of DNA and polymerase chain reaction analysis, direct sequencing method was applied to detect mutations of the *p53* gene. The *p53* mutation was detected in five of 137 samples with LULs-NDE (4%) and in five of 15 samples with dysplasia (33%). A hotspot mutation was found in 20% of LULs-NDE with *p53* mutation and in 40% of dysplasia with *p53* mutation. In contrast, no *p53* mutations were found in 103 paired NDE samples with normal Lugol staining. In biopsy samples from oesophageal cancer-free individuals, the *p53* missense mutations containing a hotspot mutation were found in NDE, which was identified as an LUL. These findings suggest that some LULs-NDE may represent the earliest state of oesophageal squamous cell carcinoma in Japanese individuals.
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Keywords: *p53* mutation; Lugol staining; oesophagitis; dysplasia; endoscopy; precursor

Molecular Diagnostics

Oesophageal squamous cell carcinoma (ESCC) is one of the most common carcinoma worldwide, with marked variation in its incidence rate among different countries, distinct geographic areas, and different ethnic groups (Parkin *et al*, 1988). Among oesophageal cancers in Japanese patients, 95% are squamous cell carcinomas (Registration Committee for Esophageal Cancer). In Western countries and Japan, heavy cigarette smoking and alcohol intake are the risk factors, whereas in the developing countries, exposure to dietary carcinogens and nutritional deficiencies are believed to be the major aetiologic factors (Yang, 1980; Von Rensburg, 1981; Parkin *et al*, 1988; Yokoyama *et al*, 1995). However, results from previous studies suggest that malignant transformation of human oesophageal epithelium is a multistage progressive process (Yang, 1980; Yang and Qiu, 1987; Qiu and Yang, 1988; Wang *et al*, 1990; Bennett *et al*, 1992; Wang *et al*, 1993; Greenblatt *et al*, 1994).

Characterisation of human oesophageal precancerous lesions at the molecular level is of critical importance to our understanding of the aetiology of ESCC and to the identification of useful biomarkers for prevention studies of that disease. Mutation analyses among high-risk Chinese populations have demonstrated that *p53* gene mutations occur at an early stage of oesophageal carcinogenesis, both in the setting of basal cell hyperplasia (BCH) and in dysplastic lesions (Bennett *et al*, 1992; Wang *et al*, 1993; Gao *et al*, 1994; Jaskiewicz and De Groot, 1994; Parenti *et al*, 1995). An early indicator of abnormality in individuals predisposed to ESCC is an increased proliferation of the oesophageal epithelial cells, morphologically manifested as BCH, dysplasia, and cancer *in situ*. Most of these lesions could be considered as precancerous lesions because of the presence of *p53* mutations (Yang, 1980; Yang and Qiu, 1987; Qiu and Yang, 1988; Wang *et al*, 1990; Mandard *et al*, 2000). But it is under debate whether BCH is a precancerous lesion for ESCC or not, as no hotspot mutations of the *p53* gene were found in BCH samples (Shi *et al*, 1999).

Although endoscopic detection for early ESCC is extremely important because of excellent 5-year survival rate (Yoshinaka *et al*, 1991; Kumagai *et al*, 1993), two-thirds of oesophageal intraepithelial carcinomas have been overlooked by conventional endoscopy alone (Sugimachi *et al*, 1989). A simple technique of spraying Lugol solution in the oesophagus is highly sensitive for identifying dysplasia and intraepithelial carcinoma (Mori *et al*,

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1993; Meyer et al, 1997; Dawsey et al, 1998). According to the Lugol staining pattern, completely 'unstained' areas were found in approximately 90% of high-grade dysplasia and carcinoma, whereas approximately 90% of staining areas, which were less intensely stained than normally stained epithelium, were non-dysplastic lesions and the remaining 10% were dysplasia (Mori et al, 1993). Therefore, Lugol-unstained lesions (LULs) are detectable not only in dysplasias and carcinomas but also in non-dysplastic areas, for example with oesophagitis, or in the setting of Barrett's oesophagus (Sugimachi et al, 1989; Dawsey et al, 1998). In contrast, Lugol staining methods were not used in most studies regarding *p53* mutational status in oesophageal precancerous lesions such as dysplasia, BCH, and esophagitis (Yang and Qiu, 1987; Qiu and Yang, 1988; Wang et al, 1990; Bennett et al, 1992; Wang et al, 1993; Gao et al, 1994; Greenblatt et al, 1994; Jaskiewicz and De Groot, 1994; Parenti et al, 1995; Shi et al, 1999; Mandard et al, 2000).

Resected specimens from cancer patients were used in the analysis of the *p53* mutations in the previous studies (Yang, 1980; Yang and Qiu, 1987; Qiu and Yang, 1988; Wang et al, 1990; Shi et al, 1999; Mandard et al, 2000). Little information is available regarding the *p53* mutational status in the Lugol-unstained lesions with non-dysplastic epithelium (LULs-NDE) of oesophageal cancer-free subjects. To determine the genetic alterations in the early stage of oesophageal carcinogenesis, oesophageal cancer-free subjects should be selected. Endoscopic detection of oesophageal precancerous lesions and molecular diagnosis is of clinical importance to identify high-risk patients and to prevent the development of ESCC. We carried out a prospective study of the *p53* mutational status of both LULs-NDE and paired samples of normal Lugol staining areas from endoscopic biopsy samples obtained after spraying the oesophagus with Lugol solution.

MATERIALS AND METHODS

Study design

To investigate whether LULs-NDE were related to the carcinogenesis of oesophageal squamous epithelium or not, the *p53* mutational status in LULs-NDE was analysed prospectively. Secondary end points were to elucidate whether BCH is related to oesophageal carcinogenesis through the *p53* mutational status and examine malignant potential in multiple LULs. Recruited subjects were composed of oesophageal cancer-free individuals who visited our hospital for a health checkup between April 1999 and March 2001. Subjects were recruited on the basis of the following criteria: male and female individuals, age in the range of 20–80 years, the subjects performance status being 'zero' according to Eastern Cooperative Oncology Group (ECOG), and the subjects with no symptoms of dysphagia, abdominal pain, chest and/or back pain, or vomiting were eligible. As LULs can be caused by reflux oesophagitis, subjects with heartburn and those receiving proton pump inhibitor therapy were excluded. Subjects who had active malignant disease, and who had undergone oesophagectomy or chemoradiotherapy for ESCC, were excluded. After endoscopic observation, subjects who had oesophageal varices, Barrett's oesophagus, or reflux oesophagitis were also excluded. Although heavy cigarette smoking and alcohol intake are the major risk factors of ESCC, whether the oesophageal precancerous lesions are caused by such daily consumption or not is uncertain. Therefore, the subjects were not selected based on risk factors such as smoking and alcohol drinking. Participants were interviewed using structured questionnaires, which included queries about smoking and drinking status after recruitment. All subjects gave informed consent for participation in the study. The study protocol was approved by the Human Ethics Review Committee of Showa University School of Medicine.

Patient population

A total of 599 subjects were recruited: 542 subjects matched the recruitment criteria and 42 subjects were excluded from the study. The reasons for exclusion were symptom-free reflux oesophagitis in 15 subjects, Barrett's oesophagus in five subjects, gastric carcinoma in one subject, and rejection to the study in 21 subjects. The mean age was 61 years, ranging from 20 to 80 years, and the male to female ratio was 274/268. Of the 542 subjects, 157 (29%) and 130 (24%) had daily consumption of cigarette and alcohol, respectively.

Endoscopic examination

Videoendoscopy (Q240, Olympus, Tokyo, Japan) following Lugol solution spraying was performed on all oesophageal cancer-free subjects who matched the recruitment criteria. After ordinary endoscopic observation, 5–10 ml of 2.0% glycerin-free Lugol's iodine solution, which was a brown liquid consisting of 2.0 g potassium iodine and 4.0 g iodine in 100 ml distilled water, was sprayed from the gastroesophageal junction to the upper oesophagus using a plastic spray catheter (washing tube PW-5L; Olympus, Tokyo, Japan) passed through the biopsy channel of the endoscope. The whole oesophagus was observed again and epithelial areas were categorised as unstained, normally stained, or overstained. We defined LULs as those areas either staining less intensely than normally stained epithelium or completely unstained (Figure 1A–C); this group of lesions included carcinoma, dysplasia, and oesophagitis. When 10 and more than 10 LULs were detected in one endoscopic view, we defined them as multiple LULs (Figure 1D). Biopsies were taken under endoscopic guidance for LULs and paired normal Lugol staining background epithelium. The background epithelium specimens were obtained 1–5 cm away from LULs. We confirmed that samples were correctly taken from LULs during endoscopic observation. Histologic diagnosis among normal epithelium, oesophagitis, BCH, dysplasia, and carcinoma was made according to previously described definitions (Dawsey et al, 1994). Histologic features were evaluated by a pathologist in our hospital.

DNA extraction

Ten 2-μm-thick sections were obtained from each archival block of formalin-fixed and paraffin-embedded dysplastic and non-dysplastic tissue. One section of each block was stained with haematoxylin and eosin. The percentage of neoplastic cells was estimated by light microscopic evaluation, and the samples containing a minimum of 60% dysplastic cells were chosen. DNA samples were extracted by the ethanol/xylene method from the remaining nine sections (Goelz et al, 1985).

Analysis of the *p53* gene

Specimens were mixed with 50 μl of digestion buffer (0.04% proteinase K, 10 mM Tris-HCl at pH 8.0, 1 mM EDTA, and 1% Tween 20) and incubated at 37°C for 18 h. The DNA fragments were analysed for mutations in *p53* exons 5, 6, 7, and 8, as described in our previous report (Makino et al, 2000). Primers used for polymerase chain reaction (PCR) amplification of the *p53* gene were as follows: for exon 5, 5′-TTCACTTGTGCCCTGATTTC-3′ and 5′-CTCTCCAGCCCCAGCTGCTC-3′; for exon 6, 5′-ATTCCTCACTGATTGCTCC-3′ and 5′-TCCTCCCAGAGACCCCAGTT-3′; for exon 7, 5′-ACAGGTCCTCCCCAAGGCGCA-3′ and 5′-TGTGCAGGGTGGCAAGTGGCT-3′; for exon 8, 5′-GTAGGACCTGATTTCCTTACTGCC-3′ and 5′-CTTGGTCTCCTCCACCGCTTCTTG-3′. Polymerase chain reaction conditions were set as described in our report (Makino et al, 2000). The PCR products were purified and directly sequenced using a 3100 sequencing machine (Applied

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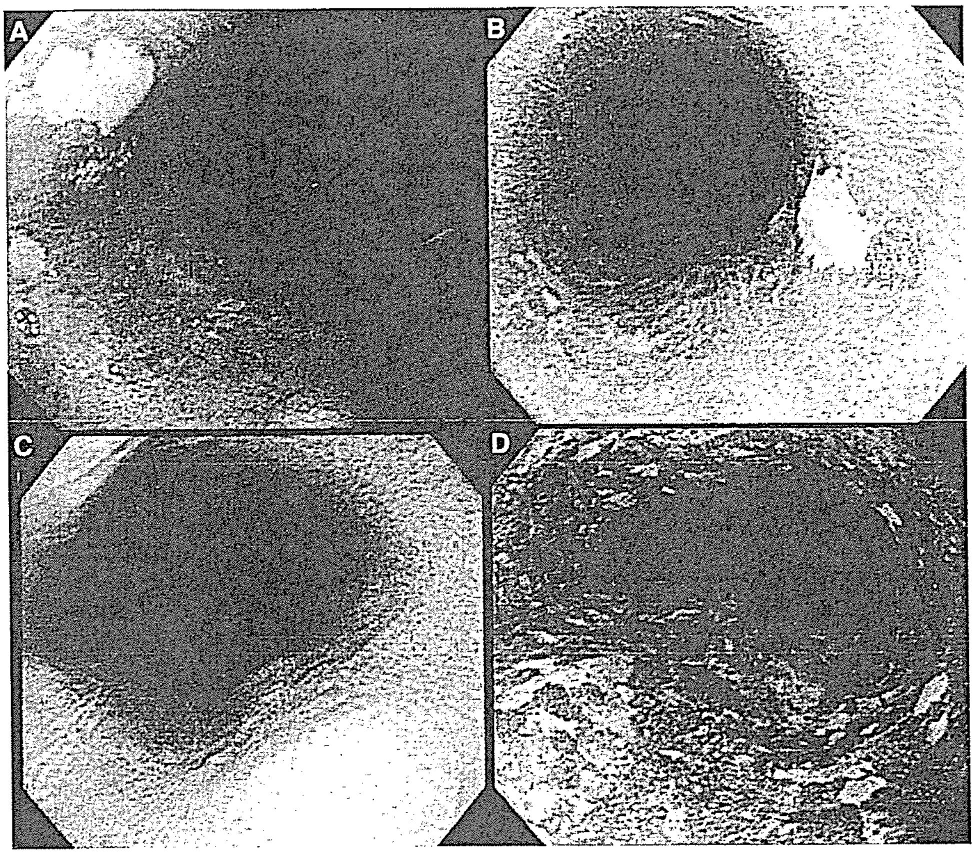

Figure 1 (**A**) Endoscopic findings of a Lugol-unstained lesion. This lesion was completely unstained. The lesion was oval and 4 mm in diameter. (**B**) Endoscopic findings of a Lugol-unstained lesion. This lesion was completely unstained. The lesion was irregular in shape and 6 mm in diameter. (**C**) Endoscopic findings of normal Lugol staining epithelium without a Lugol-unstained lesion. (**D**) Endoscopic findings of multiple Lugol-unstained lesions. Many irregular lesions that were stained less intensely than normal Lugol staining epithelium were located in one endoscopic view.

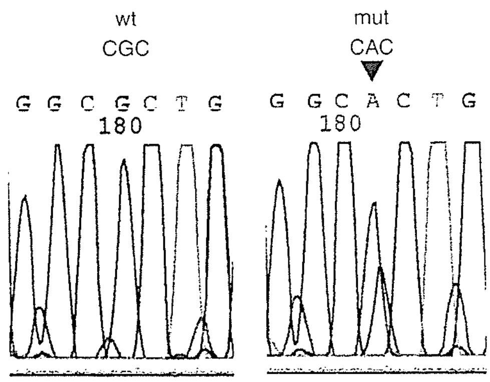

Figure 2 Mutation of the *p53* gene at codon 175 in exon 5 was shown in electropherograms. Base changed from CGC to CAC (black arrow). wt, wild type; mut, mutation.

Biosystems, Foster City, CA, USA). Peak patterns were analysed using Sequencing Analysis Software (Applied Biosystems, Foster City, CA, USA), and mutations and amino-acid changes were identified (Figure 2). To ensure reproducibility of our data, direct sequencing was performed at least twice in DNA samples.

Statistical analysis

As LULs were found in approximately 20% of 1000 patients undergoing routine endoscopy in our previous experience, sample size was estimated to be 500 patients to collect at least 100 patients with LULs. To avoid bias, the data regarding the detection of *p53* mutation in LULs and their paired normal Lugol staining areas were re-identified for genetic and clinicopathologic analyses. These data were then matched after the genetic and clinicopathologic analyses were completed. The significance of differences between the two groups was assessed by the χ^2 test or Wilcoxon rank-sum test. *P*-value of less than 0.05 was considered significant.

RESULTS

Characteristics of subjects

Out of 542 subjects, LULs were found in 103 (19%).The mean age was 62 years, ranging from 25 to 80 years, and the male to female ratio was 63/50. Of the 103 subjects, 35 (34%) and 31 (30%) had a daily habit of cigarette smoking and alcohol drinking, respectively. No significant difference in the frequency of daily cigarette smoking ($P = 0.213$) or alcohol ($P = 0.107$) consumption was seen between the subjects with LULs and those without.

Histologic and clinicopathologic findings

The samples of LULs consisted of 137 NDE and 15 dysplastic samples, whereas no dysplastic samples were detected in the normal Lugol staining samples (Table 1). Whereas the histologic finding in all samples of LULs-NDE was oesophagitis, 78% of the 103 normal Lugol staining epithelium samples were oesophagitis ($P < 0.0001$). The histologic grade of dysplastic samples was low-grade in nine of 15 (60%) samples and high-grade in six (40%) samples.

The clinicopathologic findings of LULs-NDE and dysplasia are shown in Table 2. Most LULs-NDE and dysplasia also were located in the middle third of the thoracic oesophagus, as most invasive ESCCs were located in the same portion (Registration Committee for Esophageal Cancer). The characteristics of LULs-NDE were minute size (<5 mm in diameter), oval shape, and location in the middle third of the oesophagus.

Table 1 Histologic findings of biopsy samples from 103 oesophageal cancer-free patients

	Lugol-unstained lesions	Normal Lugol staining epithelium	P-value
Number of samples	152	103	
Histologic findings			
Dysplasia	15	—	
Oesophagitis	137	80	<0.0001
Normal epithelium	—	23	
Basal cell hyperplasia			
Present	3	2	0.986
Absent	149	101	

Table 2 Clinicopathologic characteristics and presence of *p53* mutation of LUL-NDE and dysplasia

	LUL-NDE (n = 137)	Dysplasia (n = 15)	P-value
Mean size (mm)	4	9	0.032
Range (mm)	1–6	5–20	
Shape of LUL			
Oval	108	5	<0.0001
Irregular	29	10	
Location			
Upper third	19	1	0.441
Middle third	90	9	
Lower third	28	5	
P53 mutation			
Present	5	5	<0.0001
Absent	132	10	
Hotspot mutation (10 samples with p53 mutation)			
Present	1	2	0.490
Absent	4	3	

LUL-NDE = Lugol-unstained lesion with non-dysplastic epithelium; location = location of the oesophagus.

Mutation of the *p53* gene

p53 mutation was detected in five of the 137 LULs-NDE samples, whereas no *p53* mutations were found in normal Lugol staining epithelium samples (Table 2). The mutations of the *p53* gene in LULs-NDE were one in exon 6, three in exon 7, and one in exon 8, and all were missense mutations (Table 3). A 'hotspot' mutation at codon 273 was found in one of the five LULs-NDE. A *p53* mutation was found in three of nine subjects (33%) with low-grade dysplasia and two of six subjects (33%) with high-grade dysplasia. The mutations of the *p53* gene in dysplastic lesions were three in exon 5 and two in exon 6, and four were missense mutations and one was a nonsense mutation resulting in insertion of a stop codon. A hotspot mutation at codon 175 was found in two of five dysplasia samples and these two samples were low-grade dysplasia.

In contrast, 22 (16%) of 137 LULs-NDE showed squamous atypia (Table 4). *p53* mutation was found in one (4.5%) of 22 LULs-NDE with squamous atypia (Figure 3A), and in four (3.5%) of 115 LULs-NDE without squamous atypia (Figure 3B and Table 4). Approximately 80% of the normal Lugol staining epithelium samples were oesophagitis, whereas no squamous atypia was found in normal epithelium samples.

Basal cell hyperplasia was present in 2% of LUL samples and 2% of normal Lugol staining epithelium samples alone (Table 1). Notably, *p53* mutations were not found in both LULs-NDE and normal Lugol staining epithelium samples with BCH (Table 4).

Multiple LULs

A single or few LULs were detected in 98 (95%) subjects and multiple LULs were found in five (5%) subjects (Table 5). Although multiple LULs were found in only five (0.9%) of the 542 subjects, three of the five subjects with multiple LULs (60%) had dysplasia ($P = 0.003$; Table 5).

No significant difference was seen in the occurrence of *p53* mutations between subjects with single or few LULs and multiple LULs (Table 5). Although the same mutation in exon 6 at (codon 218) was found in both dysplasia and LULs-NDE in case 2 with multiple LULs-NDE (Table 3), the two lesions were independent and were not contiguous. One lesion was located in the middle oesophagus, with dysplasia of 6-mm diameter area; the other was located in the lower oesophagus, with LUL-NDE of 4-mm diameter area.

Table 3 Mutation of the *p53* gene in patients with Lugol-unstained lesions

Case	Age/sex	Histology	Size (mm)	p53	Ex	Codon	BC	AAC
1	52/M	itis	3	P	7	242	TGC→TCC	K→S
2	53/M	sev, dys.	6	P	6	218	GTG→GAG	V→E
		itis	4	P	6	218	GTG→GAG	V→E
		itis	3	A				
3	78/M	mod, dys.	0	P	6	192	CAG→TAG	*
4	71/M	mild, dys.	8	P	5	175	CGC→CAC	R→H
5	53/F	mild, dys.	6	P	5	175	CGC→GGC	R→G
6	63/M	sev, dys.	13	P	5	184	GAT→AAT	D→N
7	68/F	itis	3	P	7	241	TCC→TAC	S→Y
8	75/M	itis	4	P	8	273	CGT→TGT	R→C
		itis	2	A				
9	60/F	itis	4	P	7	239	AAC→GAC	N→D
		itis	5	A				
		itis	3	A				

itis = oesophagitis; dys, dysplasia; sev = severely; mod = moderately; P = presence of a *p53* mutation; A = absence of a *p53* mutation; EX = exon; BC = base change; AAC = amino-acid change; * = stop codon.

Table 4 Relationship between presence of *p53* mutation and BCH or squamous atypia in 137 LULs-NDE

	p53 mutation Present (n = 5)	p53 mutation Absent (n = 132)	P-value
Squamous atypia			
Present (n = 22)	1	21	0.807
Absent (n = 115)	4	111	
BCH			
Present (n = 3)	0	3	0.733
Absent (n = 134)	5	129	

LULs-NDE = Lugol-unstained lesions with non-dysplastic epithelium; BCH = basal cell hyperplasia.

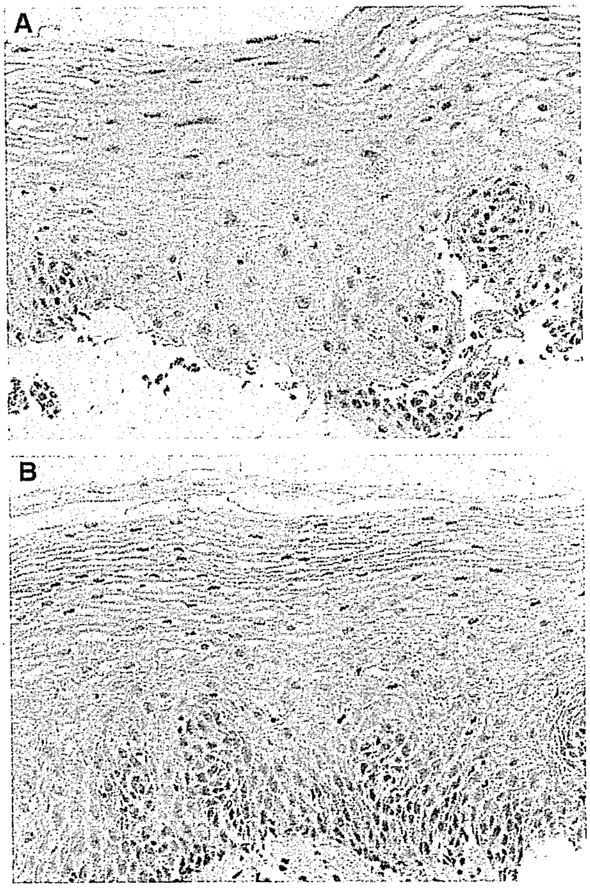

Figure 3 (**A**) Histologic findings of squamous atypia in a Lugol-unstained lesion with *p53* mutation. The region with squamous atypia was a small portion in contact with the basal cell layer. In the region, the nucleus was slightly enlarged, whereas pleomorphism and hyperchromasia were not seen. According to histological criteria of the Chinese group, the findings of slightly mononuclear enlargement having neither pleomorphism nor hyperchromasia were insufficient for diagnosis of dysplasia, and was decided as inflammation containing atypia. (**B**) Histologic findings of no squamous atypia in a Lugol-unstained lesion with *p53* mutation. Of the five Lugol-unstained lesions with non-dysplastic epithelium (LULs-NDE) containing *p53* mutation, squamous atypia was not found in four LULs-NDE.

DISCUSSION

This study is aimed to evaluate whether LULs-NDE are related to the carcinogenesis of oesophageal squamous epithelium or not, and *p53* mutational status in LULs-NDE is analysed on the basis of molecular events in the progressive process of carcinoma. As *p53* mutation is a well-known sequence in dysplasia and carcinoma, this biomarker was determined to identify the precancerous lesions in the study. The unique observation in this prospective study is that missense mutations of the *p53* gene were found in LULs-NDE, although no *p53* mutations were found in paired normally Lugol-stained non-dysplastic epithelium in subjects with LULs-NDE. The results strongly suggest that some of the LULs-NDE can progress to dysplastic lesions through *p53* alterations and support the hypothesis that some of 'Lugol-unstained non-dysplastic areas' in Japanese individuals without reflux esophagitis play an important role in oesophageal carcinogenesis.

Mutations of the *p53* tumour suppressor gene are the most common genetic abnormalities in solid human cancers (Nigro *et al*, 1989; Hollstein *et al*, 1990; Lane, 1992; Vogelstein *et al*, 2000; Vousden and Lu, 2002; Oliver *et al*, 2004). Missense mutations are found in 78% of the 6177 somatic *p53* mutations in exons 5–8 (Hussain and Harris, 1999), suggesting a correlation between the degree of evolutionary diversity and the structural or functional importance of individual amino-acid residues (Greenblatt *et al*, 1994). The change of protein structure or function caused by the individual amino-acid residues in LULs-NDE might be early molecular events in carcinogenesis. In contrast, *p53* gene mutations have been proposed to be concentrated in six hotspots (Hainaut *et al*, 1997; Hussain and Harris, 1999; Vikhanskaya *et al*, 2005). Based on the updated *p53* Gene Mutation Database containing 5961 mutations, codons 175, 245, 248, 249, 273, and 282 have been identified as mutation hotspots in human cancers, and the incidence of the hotspot mutations is specific molecular alterations in solid human cancers (Hainaut *et al*, 1997). A hotspot can identify a relationship between the mutation, protein structure and function, and carcinogenesis (Hsu *et al*, 1991; Cho *et al*, 1994; Greenblatt *et al*, 1994; Tornaletti and Pfeifer, 1994). Furthermore, hotspot mutations in carcinomas represent protein alterations that provide a selective growth advantage to the cell, and missense mutations at six hotspots account for 25–30% of the mutations (Greenblatt *et al*, 1994; Hainaut *et al*, 1997; Hussain and Harris, 1999; Ito *et al*, 2000). Therefore, protein alterations that provide a selective growth advantage to the cell would have already occurred in cells of LULs-NDE before histologic transformation into dysplastic cells. Mutations at codon 175 and 273 have been shown to have transforming frequencies that are 22- and eight-fold, respectively, the basal level of wild-type p53 protein (Zambetti and Levine, 1993). From our results, the LUL-NDE or low-grade dysplasia containing mutations with high transforming activities, such as codon 175 and 273 mutations, might have growth advantages favouring progression to invasive ESCC with the acquisition of other genetic changes, and may acquire malignant potential before morphologically manifested cell proliferation at an early molecular level of carcinogenesis.

One group has proposed that BCH is an early indicator of oesophageal carcinogenesis (Yang, 1980; Yang and Qiu, 1987; Qiu and Yang, 1988; Wang *et al*, 1990). Wang *et al* (1996) reported that BCH can be found in 69% of biopsy samples in symptom-free patients and that *p53* mutations can be found in BCH and dysplastic samples, whereas no hotspot mutations are contained in these mutations (Shi *et al*, 1999). We also identified the histologic findings of BCH in LULs-NDE and the paired normal Lugol staining area according to histologic criteria used in the Chinese group (Dawsey *et al*, 1994), whereas prevalence of BCH was low in our Japanese subjects and no *p53* mutations were found. We do not believe that the role of BCH is related to oesophageal carcinogenesis in the Japanese population. In contrast, we did not suggest that the daily cigarette or alcohol consumption was directly related to the occurrence of LULs-NDE in this study despite high risk factors in patients with ESCC.

Using Lugol solution spraying methods, as the normal squamous epithelium contains glycogen that interacts with the

Table 5 Presence of dysplasia and *p53* mutation between single or few and multiple Lugol-unstained lesions in 103 patients

	Single or few LULs (n = 98)	Multiple LULs (n = 5)	P-value
Dysplasia			
Present	12 (12%)	3 (60%)	0.003
Absent	86 (88%)	2 (40%)	
p53 mutation			
Present	8 (8%)	1 (20%)	0.361
Absent	90 (92%)	4 (80%)	

LULs = Lugol-unstained lesions.

iodine of Lugol's solution, normal epithelium of the oesophagus becomes uniformly greenish brown (Sugimach *et al*, 1991; Katagiri *et al*, 2004). Dysplastic and inflammatory epithelia of the oesophagus are not stained, as the region showing dysplasia and oesophagitis has a reduced or no glycogen content (Sugimach *et al*, 1991). Therefore, these minute lesions that were not identifiable by conventional endoscopic observation become visible when Lugol's solution is used. There is a high possibility that inflammation having a reduction in glycogen content is related to the initiation of oesophageal carcinogenesis because no squamous atypia and no *p53* mutations are found in normal Lugol staining areas with sufficient glycogen content. Squamous atypia would be transitional lesions from oesophagitis to dysplasia.

Although the prevalence of multiple LULs was low in oesophageal cancer-free subjects (0.9%), dysplasia occurred frequently in subjects with multiple LULs (60%). Muto *et al* (2002) reported that multiple LULs were found in 27% of head and neck cancer patients, and secondary ESCCs were found in 72% of such cancer patients with multiple LULs. They provided essential information about field cancerisation and malignant potential with respect to multiple LULs. The field cancerisation phenomena proposed that multiple squamous cell carcinomas occurred either simultaneously with the primary lesion (synchronous) or after a period of time (metachronous) in the oesophagus and the head and neck region. There is a possibility that widespread epithelial oncogenic alterations were found in patients with multiple LULs. In case 2, the same mutation at codon 218 was found in both LUL-NDE and dysplastic lesion, whereas *p53* mutation was not detected in background normal Lugol staining epithelium. The *p53* mutational status, in this case, reflects the phenomena of field cancerisation, which can be considered as high malignant potential.

The *p53* missense mutations containing a hotspot mutation were found in LULs-NDE in oesophageal cancer-free individuals without reflux oesophagitis. The finding suggests that LUL-NDE is an initial lesion for oesophageal carcinogenesis, and that the role of BCH is less clear for oesophageal carcinogenesis in Japanese individuals. The characteristic findings of high-risk population of oesophageal carcinoma were evaluated by genetic analyses, because it appeared that we emphasise the importance of both endoscopic detection of LUL-NDE and molecular diagnosis. We concluded that the understanding of aetiology in human oesophageal precursor at the molecular level could provide essential information about the identification of useful biomarkers for prevention studies.

ACKNOWLEDGEMENTS

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Phase I/II Study of Oxaliplatin with Weekly Bolus Fluorouracil and High-Dose Leucovorin (ROX) As First-Line Therapy for Patients with Colorectal Cancer

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Background: Infusional fluorouracil (5-FU) and leucovorin (LV) with oxaliplatin is one of the current standard regimens for the treatment of patients with metastatic colorectal cancer. Weekly bolus 5-FU with high-dose LV (Roswell Park Memorial Institute Regimen: RPMI) is the most commonly used regimen in Japan. The objectives of this study were to determine the recommended dose (RD) of RPMI combined with oxaliplatin and to evaluate the toxicity and efficacy at the RD.
Methods: The subjects were 18 patients with metastatic colorectal cancer. Oxaliplatin (85 mg/m^2) was given intravenously over 2 h on days 1 and 15 with *l*-LV (250 mg/m^2) given intravenously over 2 h and 5-FU as an intravenous bolus on days 1, 8, and 15. This treatment was repeated every 4 weeks. The dose of 5-FU was escalated from 400 mg/m^2 (level 1) to 500 mg/m^2 (level 2).
Results: A total of 14 patients received level 1, and 4 received level 2. Three of the patients had dose-limiting toxicity (DLT) in cycle 1 of level 2 (grade 3 thrombocytopenia, grade 4 neutropenia and grade 2 neutropenia in one patient each), requiring that treatment was delayed for longer than 7 days. None of the 14 patients given level 1 had DLT or grade 3 or 4 gastrointestinal toxicity. Sensory neuropathy occurred in all patients. Objective response rates were 61% in the 18 patients studied and 64% at level 1. The median time to progression was 171 days, and the median overall survival time was 603 days in the 18 patients studied.
Conclusions: Oxaliplatin (85 mg/m^2) with weekly bolus 5-FU (400 mg/m^2) and high-dose *l*-LV (250 mg/m^2) is recommended for further phase III studies in patients with metastatic colorectal cancer.

Key words: colorectal cancer – bolus 5-fluorouracil – leucovorin – oxaliplatin – RPMI

INTRODUCTION

Infusional fluorouracil (5-FU) and leucovorin (LV) with oxaliplatin is one of the current standard regimens for first- and second-line chemotherapy in patients with metastatic colorectal cancer (1–3). The combination of oxaliplatin with infusional 5-FU and LV (FOLFOX4) has been shown to be superior to infusional 5-FU plus LV (LV5FU2) and single-agent oxaliplatin in terms of response rate, median time to progression (TTP), and alleviation of tumor-related symptoms in patients with metastatic colorectal cancer who have disease progression after irinotecan with bolus 5-FU plus leucovorin (IFL, Saltz regimen) (2). Objective response rates were 9.9% for FOLFOX4, 1.3% for oxaliplatin alone and 0% for LV5FU2 ($P < 0.0001$). Median TTP was 4.6 months for FOLFOX4, 1.6 months for oxaliplatin and 2.7 months for LV5FU2 ($P < 0.0001$).

FOLFOX4 has also been evaluated as first-line therapy, and a randomized study (N9741) has shown a significantly better response rate, median TTP and median overall survival time (MST) as compared with conventional regimens (3). The response rate in patients given FOLFOX4 (45%) was higher than that in patients given IFL (31%, $P = 0.002$). Moreover, TTP was significantly longer with FOLFOX4 (8.7 months)

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than with IFL (6.9 months; $P = 0.0014$). The MST in patients treated with FOLFOX4 was 19.5 months as compared with only 15.0 months in those treated with IFL ($P = 0.0001$).

Infusional 5-FU regimens were shown by de Gramont (4) to provide a higher response rate with marginal survival benefit as compared with bolus 5-FU regimens. However, infusional 5-FU with LV has the drawbacks of increased inconvenience, cost and morbidity, related to the use of a portable infusion pump and a central venous catheter. Weekly bolus 5-FU with high-dose LV (RPMI regimen) is the most commonly used schedule in Japan and the United States, and bolus 5-FU plus low-dose LV with irinotecan (modified Saltz regimen) has been shown to have high antitumor activity with a favorable toxicity profile in Japanese patients (5–8). Single-agent oxaliplatin (130 mg/m^2) in a tri-weekly regimen has also been found to be effective and tolerable in Japanese as well as Western patients (9). Phase II studies of oxaliplatin as second-line therapy in patients with fluoropyrimidine-pretreated metastatic colorectal cancer reported objective response rates of 9–11% and an MST of 8.2–11.3 months (10–11). However, whether bolus 5-FU plus LV can be combined safely with oxaliplatin in Japanese patients remains unclear.

The primary objectives of this phase I/II study were to estimate the maximal tolerated dose (MTD) and determine the recommended dose of bolus 5-FU plus *l*-LV in combination with oxaliplatin. In the phase II part, we also evaluated the toxicity and antitumor activity of this regimen at the recommended dose.

PATIENTS AND METHODS

PATIENT ELIGIBILITY

Patients with histologically confirmed colorectal cancer who had measurable metastatic disease were eligible for the study. Prior chemotherapy and radiotherapy for metastatic disease were not permitted. Patients who had received adjuvant oral fluorouracil-based therapy were eligible if they had remained free of disease for at least 6 months after the completion of such therapy. Other eligibility criteria included an age of 20–75 years; an Eastern Cooperative Oncology Group (ECOG) performance status of 0–2; adequate baseline bone marrow function (white blood cell [WBC] count more than the lower limit of normal at each hospital and <12 000/μl, neutrophil count >2000/μl and platelet count >100 000/μl), hepatic function (serum bilirubin level 1.5 times the upper limit of normal or less, and serum aspartate aminotransferase and alanine aminotransferase 2.5 times the upper limit of normal or less) and renal function (serum creatinine level 1.5 times the upper limit of normal or less); and a life expectancy of at least 12 weeks. All patients gave written informed consent.

Patients were excluded if they had symptomatic brain metastasis; pre-existing watery diarrhea; concomitant nonmalignant disease, such as cardiac, pulmonary, renal or hepatic disease; or uncontrolled infection. This study was approved by the institutional review board of each center. Before enrollment, all patients underwent a physical examination (including documentation of measurable disease), a complete blood cell count with differential count, serum chemical analysis, chest radiography, electrocardiography, and computed tomographic (CT) scanning or magnetic resonance imaging (MRI).

TOXICITY AND RESPONSE CRITERIA

Toxicity was assessed according to the National Cancer Institute Common Toxicity Criteria, Version 2.0 (NCI-CTC) (12). Neurotoxicity was reported according to the following grading scale: grade 1, dysesthesia or paresthesia that completely regressed within 6 days; grade 2, dysesthesia or paresthesia persisting for 7 days or longer; and grade 3, dysesthesia or paresthesia causing functional impairment. During the study, all patients were evaluated weekly for signs and symptoms of toxicity. Complete blood cell counts including differential count; liver function tests; measurement of urea nitrogen, creatinine and electrolyte levels; and urinalysis were performed weekly in cycle 1 and every 2 weeks in subsequent cycles.

The response of measurable and assessable disease sites was evaluated according to RECIST (Response Evaluation Criteria in Solid Tumors) (13). Tumor dimensions were assessed by CT scanning or MRI every month to confirm response and every 2 months subsequently. Partial response (PR) was defined as more than a 30% decrease in the sum of the products of the greatest perpendicular diameters of measurable lesions, without the development of any new lesions. Stable disease was defined as a steady state of response less than a PR or as progression of <20% over the course of at least 6 weeks. Progressive disease (PD) was defined as an unequivocal increase of at least 20% in the sum of the products of the greatest perpendicular diameters of individual lesions. The appearance of new clinically significant lesions also constituted a PD.

TREATMENT PLAN

Oxaliplatin was supplied as a freeze-dried powder in 100 mg vials by Yakult Honsha Co., Ltd. (Tokyo, Japan) and was reconstituted in a solution of 5% glucose in water. The reconstituted solution was then diluted with 250 ml of 5% glucose infusion solution. Oxaliplatin was administered as a 2 h infusion every 2 weeks. The duration of infusion could be extended to 6 h in patients who had pharyngolaryngeal dysesthesia during infusion. *l*-Leucovorin (Wyeth Ltd., Tokyo, Japan) was administered at a dose of 250 mg/m^2 in 500 ml of 5% glucose solution, given as a 2 h intravenous infusion on days 1, 8 and 15 of a 28 day cycle. 5-FU (Kyowa Hakko Kogyo Co., Ltd., Tokyo, Japan) was given by bolus intravenous injection 1 h after starting the *l*-LV infusion. All patients received premedication with a 5-hydroxytryptamine-3-receptor antagonist with or without dexamethasone, given as a 30 min drip infusion before chemotherapy. Treatment cycles were repeated every 4 weeks. Treatment was routinely given on an outpatient basis, except for cycle 1 of the dose-escalation portion of the protocol (see below). Subsequent treatment was withheld until the

WBC, neutrophil,and platelet counts were >3000, 1500 and 75 000 µl, respectively, and diarrhea, stomatitis and hand-foot syndrome had resolved to grade 0 or 1. Treatment was repeated until the onset of disease progression or severe toxicity.

DOSE-ESCALATION SCHEDULE

The dose of oxaliplatin was fixed at 85 mg/m^2 and that of *l*-LV was fixed at 250 mg/m^2. 5-FU was studied in dose levels of 400 and 500 mg/m^2. A minimum of three patients were studied per dose level. Dose-limiting toxicity (DLT) was defined as any of the following findings during cycle 1: (i) a neutrophil count of <500/µl, (ii) grade 3 febrile neutropenia, (iii) a platelet count of <50 000/µl, (iv) grade 3 or 4 non-hematologic toxicity, excluding nausea, anorexia, and electrolyte imbalance according to the NCI-CTC, or (v) a longer than 1 week delay in treatment as a result of drug-related toxicity in the dose-escalation portion of the protocol. If DLT occurred in 1 of the first 3 patients assigned to a given dose level, 3 other patients were additionally assigned to receive that dose level. The MTD was defined as the dose that induced DLT during cycle 1 in at least 50% of the subjects. In the second portion of the study, the recommended dose was given to 11 other patients to confirm tolerability.

The dose was modified for each patient according to a nomogram, based on hematologic or non-hematologic toxicity. If DLT occurred, the subsequent dose of oxaliplatin was reduced to 75% of the initial dose and that of 5-FU was decreased by one dose level. If the WBC count on days 8, 15 and 22 was <3000/µl, the neutrophil count <1500/µl, or the platelet count <75 000/µl, further treatment was delayed for up to 1 week until recovery. Recombinant granulocyte colony-stimulating factor was subcutaneously injected if patients had grade 4 neutropenia or grade 3 febrile neutropenia, but prophylactic use was not allowed.

RESULTS

PATIENT CHARACTERISTICS

From March 2002 to March 2003, a total of 18 patients were enrolled. All patients received at least one cycle of the study treatment. The first 7 patients participated in the dose-escalation portion of the protocol. After identification of the MTD, 11 other patients received the recommended dose below the MTD to further evaluate the tolerability and toxicity of the study regimen. The patient characteristics are summarized in Table 1. Two patients had received adjuvant oral fluorouracil-based therapy.

TOXICITY

No DLT occurred during cycle 1 in the first 3 patients given a dose of 400 mg/m^2 of 5-FU. Two of the 3 patients initially treated with 500 mg/m^2 of 5-FU had dose-limiting myelosuppression. One patient had grade 3 thrombocytopenia, and the other had prolonged grade 2 neutropenia, requiring that

Table 1. Patient characteristics

Characteristic	Level 1 (*n* = 14)		Level 2 (*n* = 4)	
	No. of patients	(%)	No. of patients	(%)
Age (years)				
Median	60.0		67.5	
Range	37–68		55–73	
Sex				
Male	8	57	3	75
Female	6	43	1	25
ECOG performance status				
0	12	86	3	75
1	2	14	1	25
Primary tumor				
Colon	9	64	4	100
Rectum	5	36	0	0
Metastatic site*				
Liver only	8	57	2	50
Lung only	2	14	0	0
Others	4	29	2	50
No. of metastatic sites				
1	13	93	3	75
⩾2	1	7	1	25

Abbreviation: ECOG, Eastern Cooperative Oncology Group.
*Target lesion according to RECIST criteria.

Table 2. Toxicity, worst grade per patient

Level Dose of 5-FU	1 (*n* = 14) 400 mg/m^2					2 (*n* = 4) 500 mg/m^2			
	Grade					Grade			
	1	2	3	4	1–4 (%)	1	2	3	4
Anorexia	8	6	0	0	100	1	1	1	0
Nausea	8	3	0	0	79	2	1	0	0
Vomiting	4	5	0	0	64	3	0	0	0
Diarrhea	4	3	0	0	50	1	2	0	0
Stomatitis	3	2	0	0	36	0	0	0	0
Fatigue	7	1	0	0	57	2	0	0	0
Injection site reaction	8	3	0	0	79	3	0	0	0
Allergic reaction	1	1	0	0	14	0	0	0	0
Sensory neuropathy	0	14	0	–	100	0	4	0	–
Alopecia	1	0	–	–	7	2	0	–	–
Neutropenia	5	4	2	0	79	0	2	1	1
Leukopenia	2	4	0	0	43	0	4	0	0
Thrombocytopenia	6	4	0	0	71	1	1	2	0
AST elevation	5	3	0	0	57	2	0	0	0
ALT elevation	3	6	0	0	64	2	0	0	0

Abbreviations: AST, aspartate aminotransferase; ALT, alanine aminotransferase.

Table 3. Objective response

	No. of patients	PR	SD	PD	Response rate (%) (95% CI)
Level 1 5-FU 400 mg/m^2	14	9	5	0	64 (35–87)
Level 2 5-FU 500 mg/m^2	4	2	2	0	50 (7–93)
All patients	18	11	7	0	61 (36–83)

Abbreviations: PR, partial response; SD, stable disease; PD, progressive disease; CI, confidence interval

treatment was delayed for longer than 1 week. The fourth patient given 5-FU 500 mg/m^2 had grade 4 neutropenia. DLT thus comprised neutropenia and thrombocytopenia. The recommended dose was determined to be 400 mg/m^2 of 5-FU in combination with 250 mg/m^2 of *l*-LV and 85 mg/m^2 of oxaliplatin (Table 2).

Eleven patients were subsequently enrolled in the second portion of this study.

Combined with the 3 initially treated patients, a total of 14 patients received the recommended dose. The median number of administered cycles was 5.5 (range, 2–11), and the total number of cycles in the 14 patients was 74. At the recommended dose, 2 patients (14%) had grade 3 neutropenia; there was no grade 4 toxicity. The relative dose intensity was 82.5% for oxaliplatin and 84.9% for 5-FU during the first 6 cycles. The causes of treatment discontinuation at the recommended dose were PD in 8 patients, almost a complete response in 1, delayed recovery from thrombocytopenia in 2 and sensory neuropathy in 3.

Sensory neuropathy occurred in all patients. There was no neurotoxicity with functional impairment in this study. The most common types of non-hematologic toxicity were anorexia, nausea, vomiting and diarrhea. No patient had grade 3 or 4 gastrointestinal toxicity at the recommended dose. Most cases of nausea and vomiting responded to dexamethasone and granisetron or other antiemetic drugs, and good oral intake was maintained. Another mild adverse event related to treatment was injection site reactions (79%). Two patients had mild allergic reactions such as skin rash or fever, typical platinum-related reactions.

Response to Therapy

The objective tumor response was determined by an external review board. Of the 14 patients given the recommended dose (level 1) 9 had a PR, yielding a response rate of 64% (95% CI: 35–87%). One of 9 responders underwent hepatectomy following this chemotherapy. Two of the 4 patients given level 2 had a PR. In the 18 patients studied, the response rate was 61% (95% CI: 36–83%), the median time to progression was 171 days (95% CI: 142–227 days) and the median overall survival time (cut-off date: March 27, 2005) was 603 days (95% CI: 442–979 days) (Fig. 1). The 1-year and 2-year survival rates were 94 and 31%, respectively.

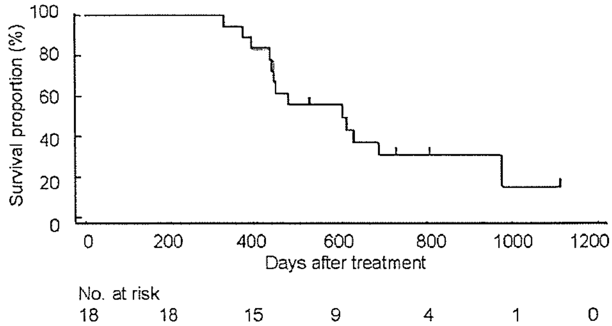

Figure 1. Overall survival in all patients.

DISCUSSION

Our results suggest that bolus 5-FU plus *l*-LV with oxaliplatin may be a safe and effective first-line treatment for metastatic colorectal cancer. The recommended dose was determined to be 400 mg/m^2 of 5-FU plus 250 mg/m^2 of *l*-LV on days 1, 8 and 15 with 85 mg/m^2 of oxaliplatin on days 1 and 15 of a 28 day cycle. DLT comprised neutropenia and thrombocytopenia at level 2. At the recommended dose (level 1), the toxicity profile was acceptable, with grade 3 neutropenia occurring in 14% of the patients; there was no other grade 3 or 4 hematologic or non-hematologic toxicity, including neurotoxicity (table3).

Two consecutive compassionate-use studies of oxaliplatin were conducted in North America until December 2000 in more than 5000 patients with metastatic colorectal cancer who had had treatment failure with at least 1 prior chemotherapy regimen (14). Patients were assigned to treatment with either single-agent oxaliplatin or oxaliplatin plus 5-FU with or without LV in various regimens. The most frequently used regimen was RPMI with oxaliplatin, received by 43–45% of the patients in both studies. Continuous infusion of low-dose 5-FU (Lokich regimen) was given to 14–20% of the patients, a modified Mayo regimen to 9–15% and LV5FU2 to only 8–10%. US and Canadian oncologists have preferred bolus regimens in combination with oxaliplatin, despite the availability of infusion schedules. The incidence of grade 3 and 4 hematologic toxicity was 17% with RPMI plus oxaliplatin and 52% with FOLFOX4 and that of grade 3 and 4 gastrointestinal toxicity was 28% with RPMI and 18% with FOLFOX4. Neurological toxicity occurred at a rate of 2% with RPMI and 8% with FOLFOX4.

Hochster et al. (15) reported the results of phase II studies of weekly bolus 5-FU (500 mg/m^2, days 1, 8 and 15, every 4 weeks) plus low-dose LV (20 mg/m^2, days 1, 8 and 15, every 4 weeks) with oxaliplatin (85 mg/m^2, days 1 and 15, every 4 weeks) (bFOL), given as first-line therapy to patients with metastatic colorectal cancer. The response rate was 63%, with a median TTP of 9.0 months and an MST of 15.9 months. Common toxicity included grade 3 and 4 neutropenia in 10% of patients, grade 3 and 4 diarrhea in 29%, and grade 3 cumulative neuropathy in 12%. Welles et al. (16) reported the results of a randomized phase II study assessing the safety and tolerability of 3 oxaliplatin-based regimens as first-line treatment for advanced colorectal cancer ('TREE 1' study). One arm was bFOL; the other 2 arms were modified FOLFOX6 (oxaliplatin 85 mg/m^2, LV 350 mg, 5-FU bolus 400 mg/m^2 and infusional 2400 mg/m^2 over the course of 46 h, every 2 weeks) and CapeOx (oxaliplatin 130 mg/m^2 on day 1 and oral capecitabine 1000 mg/m^2 twice daily for 14 days, every 3 weeks). The primary endpoint was the overall incidence of grade 3 and 4 toxicity during the first 12 weeks of each study therapy, and secondary endpoints were overall response rate and TTP. The overall incidence of grade 3 and 4 toxicity was significantly higher with modified FOLFOX6 (mFOLFOX6) (77%) than with bFOL (44%, $P < 0.001$). Moreover, mFOLFOX6 (37%) had a significantly higher incidence of grade 3 and 4 neutropenia than bFOL (14%, $P < 0.01$) and CapeOx (8%) ($P < 0.001$). Grade 3 and 4 diarrhea occurred in similar proportions of patients given bFOL (22%), mFOLFOX6 (22%) or CapeOx (25%). The overall response rate did not significantly differ among the 3 arms and was 52% (21/40) with mFOLFOX6, 38% (14/37) with bFOL and 50% (17/34) with CapeOx. Median times to discontinuation of study therapy were 5.7 months with mFOLFOX6, 4.8 months with bFOL and 4.2 months with CapeOx. These results suggested that bFOL is as active and safe as the other two regimens.

Other schedules of bolus 5-FU and low-dose LV (Mayo Clinic regimen) with oxaliplatin have also been investigated. Zori Comba et al. (17) reported the results of a phase II study of the Mayo Clinic regimen (5-FU 425 mg/m^2, days 1–5, every 4 weeks) plus low-dose LV (20 mg/m^2, days 1 to 5, every 4 weeks) with oxaliplatin (85 mg/m^2, days 1 and 15, every 4 weeks) in previously untreated patients with metastatic colorectal cancer. The response rate was 45%, with a median TTP of 3.9 months. Grade 3 and 4 neutropenia occurred in 23% of the patients, diarrhea in 34%, vomiting in 14% and stomatitis in 14%. This regimen was unacceptable because of the high incidence of severe toxicity. Ravaioli et al. (18) used the Machover scheme (5-FU 350 mg/m^2, days 1–5, every 3 weeks) and low-dose LV (20 mg/m^2, days 1–5, every 3 weeks) with oxaliplatin (130 mg/m^2, day 1, every 3 weeks) as first-line treatment for metastatic colorectal cancer. The response rate was 40%, with a median TTP of 5.9 months and an MST of 14 months. Grade 3 or severer neutropenia or diarrhea occurred in 20 and 29% of the patients, respectively. Sørbye et al. (19) performed a phase II study of Nordic bolus 5-FU (500 mg/m^2, days 1 and 2, every 2 weeks) and low-dose LV (60 mg/m^2, days 1 and 2, every 2 weeks) with oxaliplatin (85 mg/m^2, day 1, every 2 weeks) (Nordic FLOX), given as first-line therapy to patients with metastatic colorectal cancer. The response rate was 62% with a median TTP of 7.0 months and an MST of 16.1 months. Common toxicity included grade 3 and 4 neutropenia in 58% of patients, grade 3 and 4 diarrhea in 7%, and grade 3 cumulative neuropathy in 13%. Febrile neutropenia developed in 8%. That study concluded that Nordic FLOX is an effective and feasible regimen, despite the high incidence of neutropenia.

In our study, the most frequent types of non-hematologic toxicity were mild anorexia, nausea, vomiting, fatigue and diarrhea. Grade 3 neutropenia occurred in only 14% of our patients at the recommended dose. Our regimen was active and safe and may thus be a new alternative treatment for metastatic colorectal cancer. Further clinical phase II/III studies should compare RPMI plus oxaliplatin with FOLFOX to more objectively confirm our findings before our regimen is widely used clinically.

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実地臨床におけるFOLFOX療法，およびFOLFIRI療法の現状

―第一報―

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─第一報─

北信癌化学療法談話会
工藤道也[1*]・池野龍雄[2]・川手裕義[3]
熊木俊成[4]・袖山治嗣[5]・藤森芳郎[6]
宮田佳典[7]・宗像康博[8]

要　旨

長野県北部，および東部地区の一般病院における進行大腸癌に対するFOLFOX療法，およびFOLFIRI療法について約1年間の成績をまとめた。対象はFOLFOX39例，FOLFIRI15例で，初回治療としてFOLFOX療法を行った群の奏効率は50.0%（11/22）であった。FOLFOX療法によるGrade 3以上の有害事象発現率は初回治療群で19.2%（5/26），二次治療群で70.6%（12/17）。最も多かった有害事象は血液障害（白血球減少14例，血小板減少1例）であり，他に意識障害1例，食欲不振1例を認めた。Grade 3以上の末梢神経障害はなかった。一方，FOLFIRI療法によるGrade 3以上の有害事象発現率は初回治療群で60.0%（6/10），二次治療群では40.0%（2/5）であった。最も多かった有害事象は白血球減少6例であり，他にDIC 1例と脳梗塞1例を認めた。

はじめに

2005年は本邦での大腸癌化学療法に大きな変化があった。まず2月に切除不能進行・再発大腸癌に対しレボホリナート（商品名：アイソボリン®，以下ℓ-LV）・フルオロウラシル（5-FU）持続静注併用療法（de Gramont[1)]，sLV5FU2[2)]，AIO[3)] レジメン）が承認された[4)]。これによりCPT-11との併用でFOLFIRI療法[5)]が施行可能になった。さらに，4月にはオキ

1：NTT東日本長野病院外科　2：JA長野厚生連篠ノ井総合病院外科　3：JA長野厚生連新町病院外科
4：JA長野厚生連長野松代総合病院外科　5：長野赤十字病院外科　6：JA長野厚生連北信総合病院外科
7：JA長野厚生連佐久総合病院胃腸科　8：長野市民病院外科