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ORIGINAL ARTICLE

mRNA expression of RRM1, ERCC1 and ERCC2 is not associated with chemosensitivity to cisplatin, carboplatin and gemcitabine in human lung cancer cell lines

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mRNA expression of RRM1, ERCC1 and ERCC2 is not associated with chemosensitivity to cisplatin, carboplatin and gemcitabine in human lung cancer cell lines
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Background and objective: Expression of genes involved in DNA repair and/or DNA synthesis, including ribonucleotide reductase M1 (RRM1) and excision repair cross-complementation 1 (ERCC1) has been reported to be associated with chemosensitivity to platinum agents and gemcitabine. The aim of this study was to test whether similar associations would be seen between mRNA expression for the RRM1, ERCC1 and ERCC2 genes and *in vitro* chemosensitivity in lung cancer.

Methods: Using a panel of 20 lung cancer cell lines, including 15 NSCLC and 5 small cell lung cancers (SCLC), the mRNA expression levels for the RRM1, ERCC1 and ERCC2 genes were examined by quantitative real-time reverse transcription PCR. The *in vitro* cytotoxicity of cisplatin, carboplatin and gemcitabine was assessed using a tetrazolium-based colorimetric assay (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazoliumbromide (MTT) assay).

Results: Significantly, higher RRM1 mRNA expression was found in SCLC compared with NSCLC. However, there were no correlations between mRNA expression of the ERCC1, ERCC2 and RRM1 genes and chemosensitivity to cisplatin, carboplatin or gemcitabine.

Conclusions: These *in vitro* results suggest that further studies are needed to evaluate the expression of the RRM1, ERCC1 and ERCC2 genes as predictive biomarkers for sensitivity to platinum agents and gemcitabine.

Key words: chemosensitivity, DNA repair, DNA synthesis, lung cancer, predictive biomarker.

INTRODUCTION

Lung cancer is a leading cause of cancer deaths both in Japan and the USA.[1,2] Despite advances in the molecular biology, diagnosis and treatment of non-small cell lung cancer (NSCLC), which accounts for about 85% of all lung cancers, the improvement in long-term survival has only been marginal.[3] The best prospects of a cure are offered by surgical removal of early stage lung cancer, followed by concurrent chemoradiotherapy for locally advanced lung cancer. Chemotherapy for advanced lung cancer offers mild benefits in improvement of quality of life and increased survival time.

The common first-line chemotherapeutic regimens for advanced NSCLC are platinum-based combinations. The combinations of cisplatin or carboplatin with another cytotoxic agent such as paclitaxel, docetaxel, gemcitabine, vinorelbine or irinotecan produce similar response rates of about 30–40% and a median survival time of about 1 year.[4,5] To improve clinical outcomes in advanced NSCLC, clinical integration of molecular biomarkers that predict

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responses to chemotherapeutic or molecularly targeted agents, leading ultimately to individualized chemotherapy, may be important. Despite intensive studies, however, only mutations of the epidermal growth factor receptor (EGFR) gene have been validated as correlating with the clinical efficacy of EGFR tyrosine kinase inhibitors.[6]

Recently, expression of genes involved in DNA repair and/or DNA synthesis have been reported to be associated with chemosensitivity to platinum agents and gemcitabine, as well as clinical outcomes in patients with surgically resected early stage NSCLC.[7–9] Excision repair cross-complementation 1 (ERCC1) is one of the key enzymes in the nucleotide excision repair (NER) pathway.[10] Platinum agents such as cisplatin and carboplatin induce monoadducts and intrastrand or interstrand cross-linking of DNA.[10] The removal of adducts from genomic DNA is mediated by the NER pathway, in which ERCC1 forms a heterodimer with the xeroderma pigmentosum group F (XPF) protein and excises the nucleotide segment containing the adducts in coordination with XPG. ERCC2/XPD is also a component of the NER mechanism.[11] Enhanced gene expression in the NER pathway has been thought to be a major cause of resistance to cisplatin and other DNA-damaging chemotherapeutic agents. Ribonucleotide reductase M1 (RRM1) is involved in DNA synthesis, catalysing the biosynthesis of deoxyribonucleotides from the corresponding ribonucleotides, which is the molecular target of gemcitabine.[12] Earlier work had suggested that patients with low levels of tumour RRM1 mRNA expression had improved survival compared with those with high RRM1 mRNA expression levels, when treated with gemcitabine.[11] Therefore, analysis of the expression of these genes could be useful in the development of predictive biomarkers for NSCLC.

The identification of molecular biomarkers with the potential to predict treatment outcomes is essential for triaging patients to the most beneficial therapy. As one of the multiple approaches to establishing robust predictive biomarkers, we evaluated whether there would be associations between mRNA expression of the ERCC1, ERCC2 and RRM1 genes and *in vitro* chemosensitivity to cisplatin, carboplatin and gemcitabine.

METHODS

Cell lines

Fifteen NSCLC and five small cell lung cancer (SCLC) cell lines were used. Two NSCLC and 4 SCLC sell lines, with the prefix ACC-LC-, were established in our laboratories at Aichi Cancer Center. These cell lines were derived from lymph node metastases (–80, –94), pleural effusions (–49, –319) or pericardial effusions (–48, –172). NCI-H460 and A549 were purchased from the American Type Culture Collection (Manassas, VA, USA). PC-1 and PC-10 were generously provided by Dr Y. Hayata (Tokyo Medical University, Tokyo, Japan). The remaining 10 cell lines (VMRC-LCD, RERF-LC-MT, -AI, Calu1, Calu6, SK-MES-1, SK-Lu-1 and SK-LC-2, –3 and –6) were generous gifts from Dr Old and Dr M. Akiyama. All cells were cultured in RPMI-1640 medium supplemented with 10% heat-inactivated fetal bovine serum.

Drugs

Gemcitabine (Gemzar) was provided by Eli Lilly, Kobe, Japan. Cisplatin and carboplatin were provided by Bristol-Myers Squibb, Tokyo, Japan.

Cytotoxicity assay

Exponentially growing cells were harvested and resuspended at a final concentration of $1–20 \times 10^4$ cells/mL in fresh medium. Cell suspensions (100 μL) were dispensed into 96-well tissue culture plates and after 24 h at 37°C, various concentrations of the anticancer agents were added and incubated for 3 days. Cytotoxicity was evaluated by complete dose–response curves in the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazoliumbromide (MTT assay) as described previously.[13] The per cent cytotoxicity was calculated as: % cytotoxicity = {1-[Optical Density (OD) treated]/(OD control)} × 100. Each experiment was repeated at least three times. The cytotoxic effect of each treatment was assessed as the IC50 (drug concentration inducing a 50% reduction in cell survival in comparison with the control untreated cells), which was calculated from the dose–response curves.

RNA preparation

Cells were lysed with 1 mL of Isogen (Nippongene, Toyama, Japan) and total RNA was extracted according to the manufacturer's protocol, with the addition of glycogen to facilitate RNA precipitation. The RNA was further purified and treated with DNase (RNeasy kit, Qiagen, Valencia, CA, USA) according to the manufacturer's protocol, and stored at –80°C until use.

Reverse transcriptase-PCR amplification

Total RNA (50 ng) extracted from each cell line was subjected to one-step real-time reverse transcriptase (RT)-PCR for absolute quantitation of the mRNA levels of the ERCC1, ERCC2, RRM1 and β-actin genes, using the Applied Biosystems 7500F PCR system (Applied Biosystems, Foster City, CA, USA). The assays were performed in 20 μL reaction mixtures, using a One-step SYBR PrimeScript RT-PCR kit (TAKARA, Ohtsu, Japan) according to the manufacturer's protocol. The sequences of the primers are shown in Table 1. The RT-PCR condition was an initial incubation at 42°C for 5 min followed by 10-s incubation at 95°C, then 40 cycles at 95°C (5 s), 60°C (34 s). Linear regression analysis of standard curves demonstrated a strong correlation for all genes ($r^2 > 0.98$). The

Table 1 The primer sequences and PCR reaction conditions

	Forward primer sequence	Reverse primer sequence
ERCC1	CTCAAGGAGCTGGCTAAGATGT	CATAGGCCTTGTAGGTCTCCAG
ERCC2	CTGGAGGTGACCAAACTCATCTA	CCTGCTTCTCATAGAAGTTGAGC
RRM1	CGCTAGAGCGGTCTTATTTGTT	TTGCTGCATCAATGTCTTCTTT
β-actin	TTCTACAATGAGCTGCGTGTG	CAGCCTGGATAGCAACGTACA

ERCC1, excision repair cross-complementation 1; ERCC2, excision repair cross-complementation 2; RRM1, ribonucleotide reductase M1.

Table 2 IC50 values for cisplatin, carboplatin and gemcitabine in lung cancer cell lines

Cell line	Histology	Cisplatin (μmol/L)	Carboplatin (μmol/L)	Gemcitabine (μmol/L)
ACC-LC-94	Ad	1.14	18.4	0.0119
ACC-LC-319	Ad	16.5	284	>128
SK-LC-3	Ad	39.7	512	>128
A549	Ad	4.22	47	0.00821
SK-Lu-1	Ad	40.2	512	1
VMRC-LCD	Ad	14.3	147	7.17
RERF-LC-MT	Ad	5.21	92.9	>128
Calu1	Sq	9.96	89.9	0.398
SK-MES-1	Sq	1.81	28.1	0.00411
PC-1	Sq	0.127	1.84	0.00303
RERF-LC-AI	Sq	2.69	33	0.00394
PC-10	Sq	8.23	430	>128
NCI-H460	La	3.83	49.4	0.0135
Calu6	La	0.939	15.5	0.00778
SK-LC-6	La	2.35	37.3	0.00244
ACC-LC-48	SCLC	3.2	35.8	0.0191
ACC-LC-49	SCLC	3.71	52.8	1
ACC-LC-80	SCLC	3.18	43.7	0.0344
ACC-LC-172	SCLC	2.78	35.2	0.0125
SK-LC-2	SCLC	7.91	50.9	>128

Ad, adenocarcinoma; La, large cell lung cancer; SCLC, small cell lung cancer; Sq, squamous cell lung cancer.

relative gene expression levels were normalized to those of the house keeping gene, β-actin.

Statistical analysis

The strength of the association between the expression of ERCC1, ERCC2 and RRM1 and chemosensitivity of the cell lines was calculated using either Pearson's correlation coefficient or linear regression analysis. Correlations were considered significant at $P < 0.05$. One-way analysis of variance (ANOVA) followed by the Bonferroni post-test was used for comparison of RRM1 expression levels among the different cell lines. All analyses was performed using Stat View version 5.0 software.

RESULTS

Chemosensitivities to cisplatin, carboplatin and gemcitabine were examined in 20 human lung cancer cell lines, including 15 NSCLC and 5 SCLC cell lines. Cytotoxicity was measured by the MTT assay following 72 h of continuous exposure to the drugs. The IC50 values for these agents on each cell line are shown in Table 2. The IC50 values of gemcitabine for ACC-LC-319, SK-LC-3, RERF-LC-MT and PC-10 and SK-LC-2 were greater than 128 μmol/L, which was above the clinically achievable plasma concentration. There were statistically significant positive correlations between the cytotoxicities of cisplatin and carboplatin among the 15 NSCLC cell lines ($r = 0.966$; $P < 0.0001$), as well as for all 20 lung cancer cell lines, including the 5 SCLC cell lines ($r = 0.956$; $P < 0.0001$), suggesting that these agents induced similar cytotoxic effects in lung cancer cells (Fig. 1). There was a relatively weak but statistically significant correlation between the cytotoxicity of gemcitabine and that of cisplatin or carboplatin among the 15 NSCLC cell lines ($r = 0.715$; $P < 0.001$ for cisplatin, $r = 0.792$; $P < 0.001$ for carboplatin), as well as for all 20 lung cancer cell lines ($r = 0.701$; $P < 0.001$ for cisplatin, $r = 0.733$; $P < 0.001$ for carboplatin, data not shown).

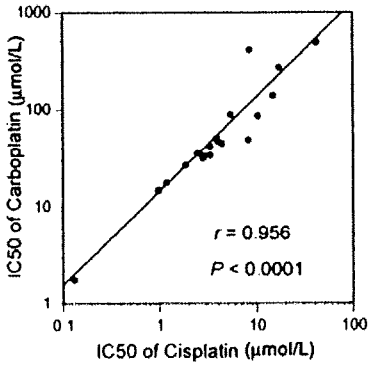

Figure 1 Correlation between chemosensitivities to cisplatin and carboplatin.

Table 3 Relative mRNA expression for ERCC1, ERCC2 and RRM1 in lung cancer cell lines

Cell line	RRM1	ERCC1	ERCC2
ACC-LC-94	1.046	1.090	1.045
ACC-LC-319	1.438	0.480	0.307
SK-LC-3	1.416	0.899	0.588
A549	1.628	0.767	0.203
SK-Lu-1	1.956	0.751	0.553
VMRC-LCD	3.291	0.744	0.671
RERF-LC-MT	1.593	0.225	0.167
Calu1	2.268	0.438	0.531
SK-MES-1	1.459	0.735	0.236
PC-1	2.889	0.749	0.713
RERF-LC-AI	3.739	0.327	0.303
PC-10	1.993	0.864	0.269
NCI-H460	2.002	0.671	0.431
Calu6	0.745	0.725	0.348
SK-LC-6	2.47	0.782	0.508
ACC-LC-48	2.388	0.414	0.257
ACC-LC-49	4.602	0.670	0.455
ACC-LC-80	3.826	1.080	0.435
ACC-LC-172	3.896	0.472	0.841
SK-LC-2	4.688	3.402	1.906

ERCC1, excision repair cross-complementation 1; ERCC2, excision repair cross-complementation 2; RRM1, ribonucleotide reductase M1.

Expression of mRNA for the ERCC1, ERCC2 and RRM1 genes was quantified by real-time PCR and normalized to β-actin mRNA expression (Table 3). mRNA expression for RRM1 was higher in SCLC cell lines compared with NSCLC cell lines. There were statistically significant differences in RRM1 expression between SCLC and adenocarcinoma, and between SCLC and large cell carcinoma (Fig. 2). There was also a statistically significant correlation between ERCC1 mRNA expression and ERCC2 mRNA expression among the 15 NSCLC cell lines ($r = 0.547$; $P < 0.05$, Fig. 3a), as well as for all 20 lung cancer cell lines ($r = 0.666$; $P < 0.005$, data not shown). However, there were no associations between RRM1 mRNA

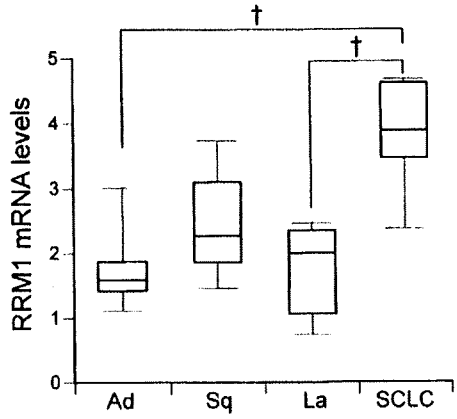

Figure 2 Predominant mRNA expression of the RRM1 gene in SCLC cell lines compared with NSCLC cell lines. Box plots show relationships between RRM1 mRNA expression and the four histological types of lung cancer. The line within each box indicates the median value. †$P < 0.005$ by ANOVA with Bonferroni correction.

expression and either ERCC1 mRNA (Fig. 3b) or ERCC2 mRNA (Fig. 3c) expression in these cell lines.

The chemosensitivity data were analysed in relation to mRNA expression of the ERCC1, ERCC2 and RRM1 genes using linear regression analysis. No significant associations were observed between the IC50 values of cisplatin, carboplatin and gemcitabine and mRNA expression for ERCC1 (Fig. 4a), ERCC2 (Fig. 4b) or RRM1 (Fig. 4c) among the 15 NSCLC cell lines. Similar results were obtained for all 20 lung cancer cell lines, including the five SCLC cell lines (data not shown).

DISCUSSION

Better and more accurate definition of the biological characteristics of the tumour is considered important for improving clinical outcome in advanced NSCLC especially in predicting response to combination chemotherapy.[14] Several reports have been published on the molecular and/or immunohistochemical analysis of molecules involved in DNA repair and/or DNA synthesis, using transbronchial and percutaneous biopsy samples from locally advanced or metastatic NSCLC.[7,11,15–17] However, there are several problems associated with mRNA and/or protein expression analyses using small tissue samples obtained by lung biopsy,[18,19] including the considerable intratumour heterogeneity, mRNA fragmentation, inevitable contamination with normal fibroblasts, the fixation procedure and storage conditions.[20] As mRNA extracted from formalin-fixed paraffin-embedded tissues is considerably fragmented, quantitative RT-PCR often yields unsatisfactory results.[21] In addition, problems with the specificity of the antibodies used for immunohistochemical analyses have been reported.[22] These limitations may result in misleading molecular

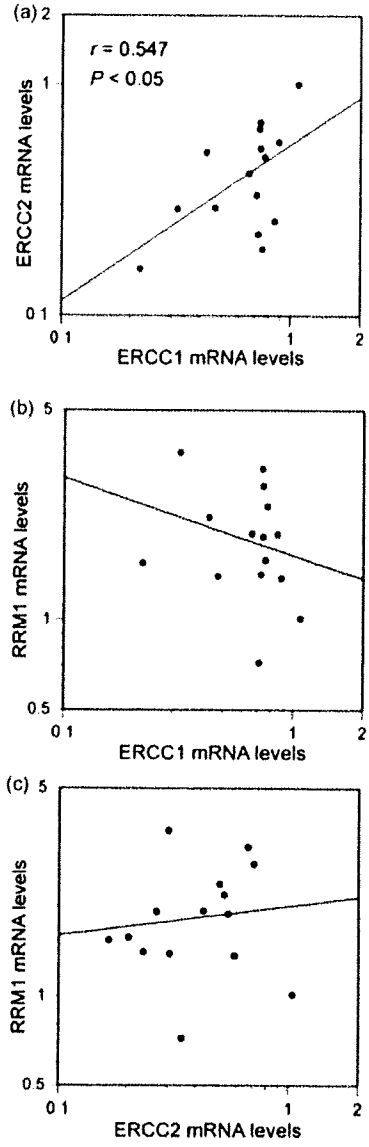

Figure 3 Correlations between (a) ERCC1 and ERCC2 mRNA expression, (b) ERCC1 and RRM1 mRNA expression and (c) ERCC2 and RRM1 mRNA expression.

analyses from clinical trials, in which the expression of biomarkers in transbronchial and percutaneous lung biopsy samples is evaluated. Thus, as one of many approaches to integrating molecular analysis with individualized chemotherapy, the *in vitro* associations between mRNA expression of the ERCC1, ERCC2 and RRM1 genes and chemosensitivity to platinum agents and gemcitabine was assessed. However, the behaviour of cell lines adapted to grow *in vitro* may differ from the *in vivo* situation, and laboratory findings may not always accurately model the clinical situation.

RRM1 expression is reported to be associated with the response to gemcitabine *in vitro.*,[23] Increased RRM1 expression has been reported in two gemcitabine-resistant NSCLC cell lines. In addition, upregulation of RRM1 has been reported in different gemcitabine-resistant cell lines,[24–26] and in a murine colon cancer model.[27] Reduced RRM1 expression has also been reported to be associated with increased sensitivity to gemcitabine in the human NSCLC H23 cell line using transfection and knockdown techniques.[7] Low levels of RRM1 expression are associated with poor survival among patients with resected NSCLC.[28] Association of increased RRM1 expression with resistance to gemcitabine was also reported in the setting of preoperative NSCLC, as well as in advanced NSCLC. In a prospective induction phase II clinical trial of chemotherapy with platinum and gemcitabine RRM1 mRNA expression was correlated with tumour response.[29] However, in the present study there was no correlation between RRM1 mRNA expression and chemosensitivity to gemcitabine, cisplatin or carboplatin. Possible explanations for the differences between this study and other *in vitro* studies are the use of tissues from different sources and the use of different assay systems, such as overexpression and/or knockdown techniques for molecular biomarkers in a limited number of cell lines. The discrepancy between this study and *in vivo* studies might be explained by possible technical limitations such as the quality of mRNA extracted from the small samples obtained by lung biopsy and the specificity of the antibody used.

The association between ERCC1 and chemosensitivity to cisplatin has been evaluated in many *in vitro* and *in vivo* studies. Increased expression of ERCC1 was associated with cisplatin resistance in ovarian cancer cells.[30] Transfection of the ERCC1 gene into an ERCC1-deficient Chinese hamster ovary (CHO) cell line conferred DNA adduct repair capability and cisplatin resistance.[31] In a human colon cancer cell line with mismatch repair deficiency, ERCC1 antisense RNA abrogated the synergistic cytotoxicity of gemcitabine and cisplatin *in vitro*.[32] The association between ERCC1 mRNA expression and chemoresponsiveness to cisplatin has been observed in primary gastric cancer and in ovarian cancer.[33–35] In the present study, there was no association between ERCC1 mRNA expression and chemoresponsiveness to either cisplatin or gemcitabine. The lack of association between ERCC1 mRNA expression and chemoresponsiveness to cisplatin is consistent with a previous *in vivo* study, of mRNA from formalin-fixed paraffin-embedded primary tumour specimens from patients with advanced NSCLC before treatment with cisplatin and gemcitabine. However, low ERCC1 mRNA expression was associated with longer survival and a trend towards a higher response rate.[16] A recent study also reported no association between ERCC1 mRNA expression and chemoresponsiveness or survival in patients with advanced NSCLC treated with platinum-based chemotherapy.[36]

ERCC1 mRNA expression in formalin-fixed paraffin-embedded tumour specimens obtained by bronchoscopic fine needle aspiration biopsy[15] is a prognostic factor in patients with resected NSCLC,[37] and patients with advanced NSCLC treated with

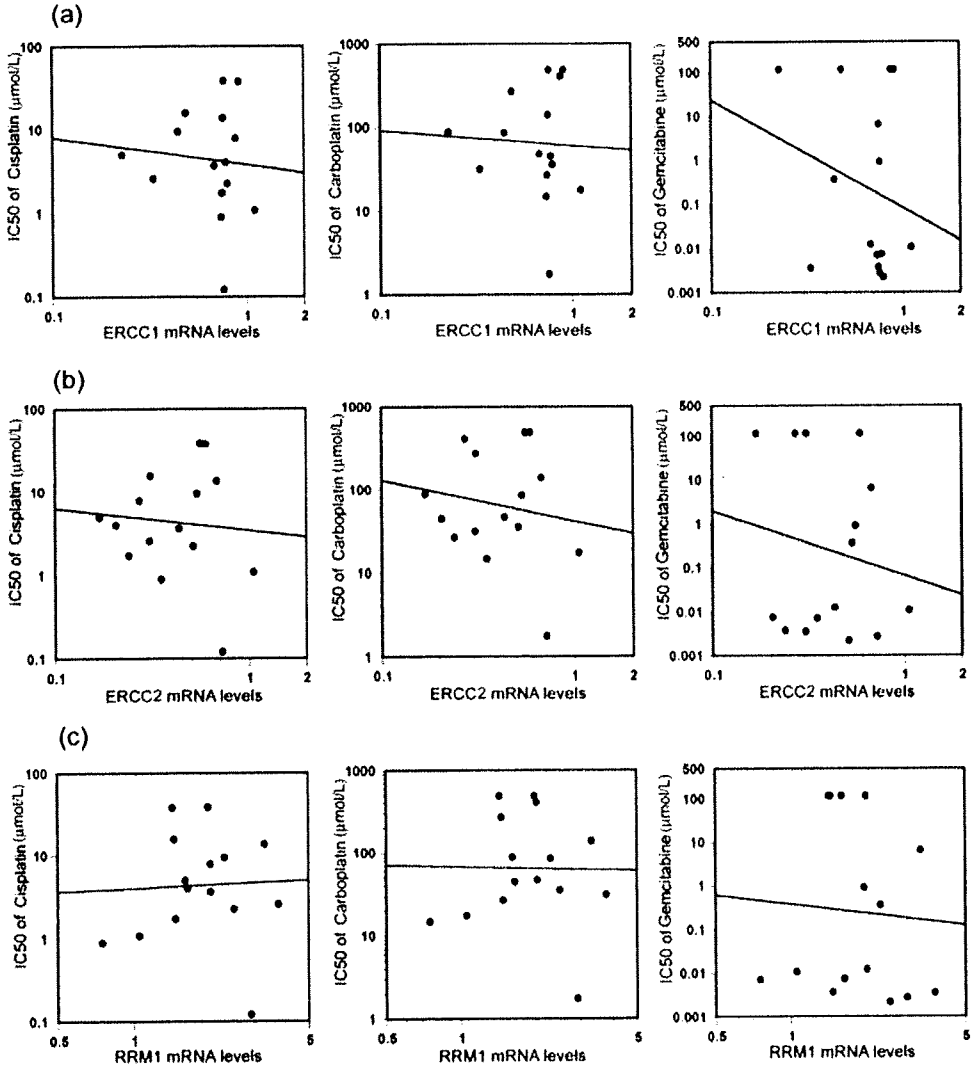

Figure 4 Associations between mRNA expression for (a) ERCC1, (b) ERCC2 and (c) RRM1 and chemosensitivities to cisplatin, carboplatin and gemcitabine.

cisplatin and gemcitabine. Furthermore, ERCC1 protein, as measured by immunohistochemical scoring, is a determinant of survival after surgical treatment of early stage NSCLC. ERCC1 protein is a prognostic factor for clinical outcome and a predictive biomarker for cisplatin-based adjuvant chemotherapy in patients with completely resected ERCC1-negative NSCLC,[8] although a problem with the specificity of the anti-ERCC1 mAb 8F1 has been reported.[22] Thus, further studies are needed to establish the role of ERCC1 in NSCLC.

The ERCC2 gene codes for a DNA helicase, which is a member of the multi-step NER pathway. The Asp312Asn polymorphism, resulting from a G/A substitution in exon 10 of the ERCC2 gene has been highly conserved through evolution, and has been reported to be a prognostic factor in patients with advanced NSCLC treated with cisplatin.[38] In addition, an *in vitro* study showed that ERCC2 overexpression leads to cisplatin resistance in a glioma cell line,[39] suggesting that expression of the ERCC2 gene may be associated with chemosensitivity to cisplatin in lung cancer cells. However, the present study failed to show associations with sensitivity to platinum agents and gemcitabine. Therefore, ERCC2 also needs further evaluation in lung cancer.

Five SCLC cell lines were included to determine whether the associations between ERCC1, ERCC2 and RRM1 mRNA expression and chemosensitivity to platinum agents and gemcitabine reported for NSCLC could be extended to SCLC. Platinum agents are key drugs and gemcitabine has modest activity in the treatment of SCLC with response rates of 11.9–13%.[40,41] However, the present study failed to show any associations. These findings are supported by a previous study, in which gene expression and the growth

inhibitory activities of various anticancer agents were similar for 19 NSCLC and 10 SCLC cell lines.[42]

There have been no *in vitro* studies examining the association between RRM1, ERCC1 or ERCC2 and chemosensitivity to platinum agents and gemcitabine, except for studies using overexpression and/or knockdown techniques. Although this *in vitro* study did not show associations in a panel of lung cancer cell lines, definitive conclusions cannot be drawn from the data, because only a limited number of cell lines were used. Exploration of the relationship between drug response phenotype and tumour genome mRNA expression profile, using cell line panels and/or tumour tissues together with cDNA and oligonucleotide arrays, would be a promising approach in the search for predictive biomarkers.[43,44] Finally, in order to validate pharmacogenetic or pharmacoproteomic candidates for lung cancer in clinical settings, further careful and more comprehensive studies using multiple approaches are warranted.

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PHASE II STUDIES

Phase II study of amrubicin in previously untreated patients with extensive-disease small cell lung cancer: West Japan Thoracic Oncology Group (WJTOG) study

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Summary *Purpose:* To evaluate the efficacy and safety of amrubicin, (+)-(7*S*, 9*S*)-9-acetyl-9-amino-7-[(2-deoxy-β-D-*erythro*-pentopyranosyl)oxy]-7,8,9,10-tetrahydro-6,11-dihydroxy-5,12-naphthacenedione hydrochloride, in previously untreated patients with extensive-disease small cell lung cancer (SCLC).

Patients and methods: A total of 35 previously untreated patients with extensive-disease SCLC were entered into the study. Amrubicin was given by daily intravenous infusion at 45 mg/m^2/day for 3 consecutive days, every 3 weeks. Unless there was tumor regression of 25% or greater after the first cycle, or 50% or greater after the second cycle, treatment was switched to salvage chemotherapy in combination with etoposide (100 mg/m^2, days 1, 2, and 3) and cisplatin (80 mg/m^2, day 1).

Results: Of the 35 patients entered, 33 were eligible and assessable for efficacy and toxicity. Of the 33 patients, 3 (9.1%) had a complete response (95% confidence interval [CI], 1.9–24.3%) and 22 had a partial response, for an overall response rate of 75.8% (95% CI, 57.7–88.9%). Median survival time was 11.7 months (95% CI, 9.9–15.3 months), and 1-year and 2-year survival rates were 48.5% and 20.2%, respectively. The most common toxicity was hematologic. Non-hematologic toxicity of grade 3 or 4 was only seen in 3 patients with anorexia (9.1%) and 1 patient with alopecia (3.0%). Salvage chemotherapy was administered to only 6 patients.

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Conclusion: Amrubicin was active for extensive-disease SCLC with acceptable toxicity. Further studies in combination with other agents for SCLC are warranted.

Keywords Amrubicin · Small cell lung cancer · Anthracycline · Previously untreated patients · Phase II study

Introduction

Small cell lung cancer (SCLC) is a major cause of cancer deaths and accounts for 15 to 20% of all lung cancers [1]. Although this cancer is initially highly responsive to chemotherapy, the vast majority of patients will ultimately relapse and die of recurrent disease within 2 years [2]. Recently, combination chemotherapy with irinotecan and cisplatin for extensive-disease SCLC produced more survival benefit than etoposide and cisplatin, the worldwide standard regimen since 1981 [3, 4]. Median survival time and 2-year survival rate of the standard regimen is 12.8 months and 19.5%, respectively. Clearly, new and more effective agents against SCLC are needed.

Amrubicin is a totally synthetic 9-aminoanthracycline, (+)-(7*S*, 9*S*)-9-acetyl-9-amino-7-[(2-deoxy-β-D-*erythro*-pentopyranosyl)oxy]-7, 8, 9, 10-tetrahydro-6, 11-dihydroxy-5,12-naphthacenedione hydrochloride, with a chemical structure similar to that of doxorubicin (Fig. 1) [5]. Amrubicin showed more potent antitumor activity than doxorubicin in several human tumor xenografts implanted in nude mice [6]. Acute toxicity of amrubicin is qualitatively similar to that of doxorubicin [7], however, amrubicin shows almost no delayed toxicity (e.g. cardiotoxicity) [8, 9].

Amrubicin is converted to an active metabolite, amrubicinol, by reduction of its C-13 ketone group to a hydroxy group. *In vitro* cytotoxic activity of amrubicinol was almost equipotent to that of doxorubicin and 20 to 220 times more potent than that of its parent compound, amrubicin [10]. Amrubicinol is considered to be closely associated with the efficacy and toxicity of amrubicin [11].

Despite their similarity in chemical structure, amrubicin has a different mode of action to doxorubicin [12]. Amrubicin and its active metabolite, amrubicinol, are inhibitors of DNA topoisomerase II. Amrubicin and amrubicinol exert cytotoxic effects by stabilizing topoisomerase II-mediated cleavable complexes, while doxorubicin does not inhibit this step of the catalytic cycle of topoisomerase II at concentrations for which it demonstrates cytotoxicity. Doxorubicin is a potent DNA intercalator, and its cytotoxicity is thought to be mainly due to this. Amrubicin and amrubicinol are about one-tenth weaker DNA intercalators than doxorubicin. Therefore, they are similar to etoposide in terms of inhibition of topoisomerase II by stabilizing the cleavable complexes, although etoposide does not show any DNA intercalating activity.

In a phase I–II study in patients with non-small cell lung cancer, amrubicin was administered as a 5-min intravenous infusion for 3 consecutive days [13]. The maximum tolerated dose (MTD) was 50 mg/m^2/day and the dose-limiting toxicities were leukopenia, neutropenia, thrombocytopenia, and gastrointestinal complications. The recommended dose for the phase II study was 45 mg/m^2/day for 3 consecutive days every 3 weeks.

Based on these experimental data and preliminary clinical reports indicating that amrubicin may be active against lung cancer, the West Japan Thoracic Oncology Group (WJTOG) evaluated it for use in SCLC. The WJTOG conducted a phase II study in previously untreated extensive-disease SCLC patients as a first-line therapy. Salvage chemotherapy with etoposide and cisplatin and an early cessation rule were set in place as precautionary measures.

Patients and methods

Eligibility criteria

Eligibility criteria included histologically or cytologically proven small cell lung cancer with extensive-disease defined as distant metastasis and/or disease involving the

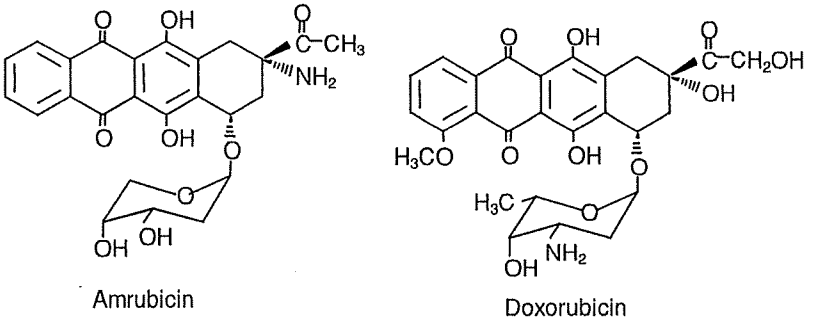

Fig. 1 Chemical structures of amrubicin and doxorubicin

contralateral hilar lymph nodes; no prior treatment; life expectancy of at least 2 months; the Eastern Cooperative Oncology Group (ECOG) performance status of 0 to 2; at least one bidimensionally measurable lesion; age less than 80; adequate organ function, such as white blood cell (WBC) count of 4000×10^6/L or greater, hemoglobin level 10 g/dL or greater, platelet count 100×10^9/L or greater, AST and ALT less than 100 IU/L, bilirubin level 1.5 mg/dL or less, creatinine concentration 1.2 mg/dL or less, electrocardiogram (ECG) findings within normal range, and left ventricular ejection fraction (LVEF) of echocardiogram 60% or greater. All patients gave written informed consent. Ineligibility criteria were: brain or bone metastases requiring radiation; continuous long-term treatment with non-steroidal anti-inflammatory drugs and glucocorticoids; pulmonary fibrosis; serious complications and other active malignancy; or pregnant or nursing subjects.

This study was approved by the institutional review boards at each participating center.

Study design

Amrubicin (Sumitomo Pharmaceuticals Co., Ltd, Osaka, Japan) was dissolved in 20 mL normal saline and administered once intravenously as a 5-min infusion at a dose of 45 mg/m^2/day on days 1 to 3, every 3 weeks.

Before treatment, all patients underwent a medical history, physical examination, hematology and serum biochemistry tests, urinalysis, ECG, LVEF, and baseline tumor measurements (chest radiography, CT scans, bone scintigraphy, and other measurements as appropriate). All measurable and assessable lesions were evaluated within 2 weeks before treatment. ECG and LVEF were undertaken within 1 month before treatment.

Complete and differential blood cell counts, platelet counts, hematocrit analysis, biochemical analysis including AST, ALT, alkaline phosphatase, LDH, total bilirubin, BUN, creatinine, serum bilirubin, albumin, total protein, and electrolyte levels (Na, K, Cl, and Ca), and urinalysis (including protein, glucose, urobilinogen, and occult blood) were performed weekly as a rule. When severe myelosuppression was observed, complete and differential blood cell counts plus platelet counts were performed 2 times or more per week. ECG was undertaken every treatment cycle and LVEF every other cycle. Chest radiography and CT scans were carried out every cycle as a rule.

Subjective and objective symptoms were observed and recorded as appropriate.

Dose modifications were made according to WBC and platelet counts. If the WBC count nadir was lower than $1{,}000 \times 10^6$/L for 4 days or longer and/or the platelet count nadir was lower than 50×10^9/L, a dose reduction of 5 mg was stipulated in the subsequent treatment course. Treatment was postponed until the WBC and platelet counts recovered to $\geq 3{,}000 \times 10^6$/L and $\geq 100 \times 10^9$/L, respectively.

In patients who demonstrated tumor regression of 25% or greater after the first course of chemotherapy, amrubicin treatment was continued. After the second course, patients had to have achieved tumor regression of 50% or greater to continue to receive the drug up to a maximum of 6 courses. Treatment of combination chemotherapy with etoposide (100 mg/m^2 on days 1, 2, and 3) and cisplatin (80 mg/m^2 on day 1) was recommended for patients who failed to fulfill any of the above criteria.

Evaluation of response and toxicity

Response was assessed according to the "Criteria for the evaluation of the clinical effects of solid cancer chemotherapy" of the Japan Society for Cancer Therapy [14], which are virtually identical to those of the World Health Organization [15]. A complete response (CR) was defined as disappearance of all lesions for a minimum of 4 weeks. A partial response (PR) was defined as a 50% or greater decrease in the sum of the products of the diameters of measurable lesions for a minimum period of 4 weeks and no new lesions. No change (NC) was defined as a decrease in the tumor mass of less than 25% or any increase of less than 25%. Progressive disease (PD) was defined as an increase in the size of any measurable lesion by 25% or greater or the appearance of new lesions.

Toxicity grading was recorded based on the side effect record form in the "Criteria for the evaluation of the clinical effects of solid cancer chemotherapy" of the Japan Society for Cancer Therapy [14].

Statistical analyses

The estimated sample size was 30 to guarantee that the lower limits of 95% confidence interval would be at least 20% at 40% of expected response rate. An early cessation rule was in place to terminate the study if at least 4 responses had not been seen among 15 patients evaluated. Median overall survival was estimated using the product-limit (Kaplan-Meier) method [16].

Results

Patient characteristics

Of 35 patients entered into this study between May 1995 and January 1997, 33 patients were eligible and assessable for efficacy and toxicity. There were 2 ineligible patients because of serious complications before treatment (cardiac

Table 1 Patient characteristics

Patient characteristics	No. of patients ($N = 33$)	%
Age (years)		
Median	66	
Range	42–78	
Sex		
Male	29	87.9
Female	4	12.1
Performance status (ECOG)		
0	5	15.2
1	26	78.8
2	2	6.1
Stage		
IIIB	1	3.0
IV	32	97.0
Prior therapy		
No	33	100

ECOG: Eastern Cooperative Oncology Group.

failure and aggravation of hepatitis, respectively), and they did not receive amrubicin. Characteristics of the 33 eligible patients are shown in Table 1. Of the 33 patients, 13 (39%) were 70 years of age or older, 88% were male, and 94% had an ECOG performance status of 0 or 1.

Efficacy

Response to amrubicin is shown in Table 2. The early cessation rule was not imposed to terminate the study, as 10 responses were seen after 15 patients were enrolled. Of 33 patients, 3 achieved a complete response, giving a CR rate of 9.1% (95% CI, 1.9–24.3%), and 22 a partial response, for an overall response rate of 75.8% (95% CI, 57.7–88.9%). Of 7 patients, 6 experiencing no change under amrubicin treatment were switched to salvage chemotherapy. Of these, 2 had partial responses and the others had no change.

The overall survival curve is shown in Fig. 2. Median survival time was 11.7 months (95% CI, 9.9–15.3 months), and 1-year and 2-year survival rates were 48.5% (95% CI, 31.4–65.5%) and 20.2% (95% CI, 6.4–34.4%), respectively.

Toxicity

The major observed toxicity was hematologic, as shown in Table 3. All patients experienced leukopenia and neutropenia. Grade 3 or 4 leukopenia occurred in 51.5% of patients and grade 3 or 4 neutropenia in 84.8%. Anemia and thrombocytopenia were observed in 78.8% and 39.4% of patients, respectively, both with a frequency of grade 3 or 4 of 21.2%. Despite the severe hematologic toxicity of amrubicin, there was no febrile neutropenia or treatment-related death during the entire treatment of 33 patients. Granulocyte colony-stimulating factor (G-CSF) was used in 55 (40%) of a total of 136 cycles, in 13 patients (39%). Most hematologic toxicity in this trial was well-controlled without dose reduction: 88% of the total treatment cycles were delivered at the planned dosage of amrubicin, 45 mg/m^2/day.

Non-hematologic toxicities observed in more than 10% of patients were anorexia (54.5%), nausea and vomiting

Table 2 Response to amrubicin

No. of assessable patients	Response (No. of patients)				CR rate, % (95% CI)	Response rate, % (95% CI)
	CR	PR	NC	PD		
33	3	22	7	1	9.1 (1.9–24.3)	75.8 (57.7–88.9)

CR: complete response; PR: partial response; NC: no change; PD: progressive disease; 95% CI: 95% confidence interval.

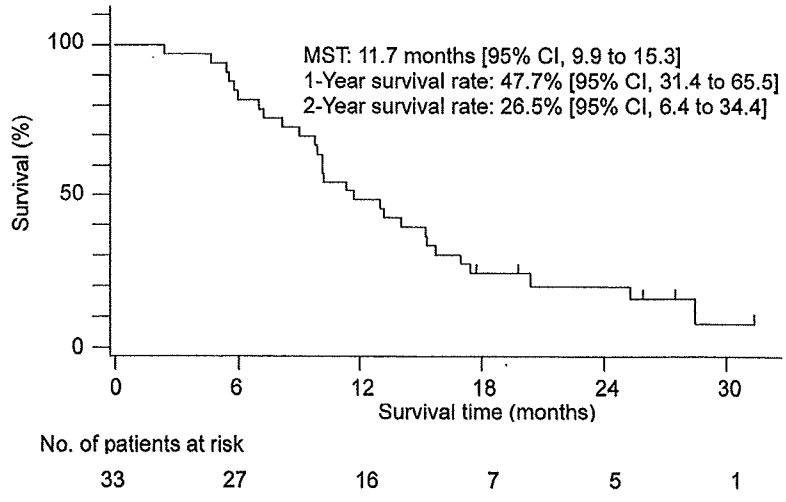

Fig. 2 Overall survival of patients with extensive-disease small cell lung cancer treated with amrubicin. MST: median survival time; 95% CI: 95% confidence interval

Table 3 Main treatment-related toxicity of amrubicin

Toxicity	No. of assessable patients	Toxicity grade others					
		1	2	3	4	≥1	≥3
		(No. of patients)				Frequency (%)	
Hematologic toxicity							
Anemia (hemoglobin)	33	12	7	6	1	78.8	21.2
Leukopenia	33	5	11	13	4	100	51.5
Neutropenia	33	1	4	14	14	100	84.8
Thrombocytopenia	33	3	3	1	6	39.4	21.2
Non-hematologic toxicity							
Stomatitis	33	2	1	0	0	9.1	0
Anorexia	33	12	3	3	–[a]	54.5	9.1
Nausea and vomiting	33	12	7	0	–[a]	57.6	0
Diarrhea	33	6	0	0	0	18.2	0
Fever	33	3	7	0	0	30.3	0
Phlebitis	33	1	1	0	0	6.1	0
Alopecia	33	11	8	1	–[a]	60.6	3.0
Total bilirubin elevation	33	1	1	0	0	6.1	0
AST elevation	33	5	0	0	0	15.2	0
ALT elevation	33	8	1	0	0	27.3	0
ALP elevation	33	1	0	0	0	3.0	0
BUN elevation	33	2	0	0	0	6.1	0
Others[b]	Headache, 1/33[c]; Rash, 1/33; Constipation, 1/33; Interstitial pneumonia, 1/33; Rhinorrhagia, 1/33; ECG abnormality, 3/32						

AST: aspartate aminotransferase; ALT: alanine aminotransferase; ALP: alkaline phosphatase; BUN: blood urine nitrogen; ECG: electrocardiogram.

[a]Toxicity grade was not defined for these toxicities.

[b]Toxicities which were not graded.

[c]Proportion of number of reported patients to number of observed patients.

(57.6%), diarrhea (18.2%), fever (30.3%), alopecia (60.6%), AST increase (15.2%), and ALT increase (27.3%). Most of these were mild (≤grade 2), with only 3 patients (9.1%) experiencing grade 3 anorexia and 1 patient grade 3 alopecia (3.0%). A single patient developed interstitial pneumonia after the second cycle of treatment; however, it was reversibly recovered by steroid therapy and cessation of amrubicin treatment. ECG abnormality was observed in 3 patients (9.4%; supraventricular extrasystole, prolonged QT interval, and T wave flattening in 1 patient each), which did not need any treatment. No LVEF decrease was observed.

Discussion

Results of this phase II study demonstrate that amrubicin is an extremely active agent against extensive-disease SCLC. The complete response rate was 9.1% (95% CI, 1.9–24.3%), overall response rate 75.8% (95% CI, 57.7–88.9%), and median survival time 11.7 months (95% CI, 9.9–15.3 months). These results are comparable or even superior to those of the standard combination regimen of cisplatin and etoposide, used as the gold standard of extensive-disease SCLC therapy since 1981 and remaining unchanged over the last 2 decades [4].

SCLC is sensitive to cytotoxic anticancer agents. Of anticancer drugs developed before 1990, a number of agents with response rates of 20% or greater for SCLC were listed as active drugs [17]. Of these drugs, etoposide, cisplatin, carboplatin, doxorubicin, cyclophosphamide, and vincristine, are still currently used as important constituents of combination regimens in the treatment of SCLC. In addition, several drugs with significant activity for SCLC have been developed since 1990. Irinotecan showed a response rate of 33% to 47% even in previously treated patients who are generally less sensitive to chemotherapy [18, 19]. Recently a new combination regimen of irinotecan plus cisplatin was demonstrated to be significantly superior to standard regimen of etoposide plus cisplatin in median survival time (12.8 months vs. 9.4 months, $P = 0.002$) [3]. In addition, topotecan, paclitaxel, docetaxel, and gemcitabine are reported to have response rates of 26% to 41% for extensive-disease SCLC patients without previous treatment [20–24]. Compared to these agents, amrubicin demonstrated a much higher response rate (75.8%) in this study, indicating it is a promising novel agent with potential to overcome the therapeutic plateau of SCLC.

The major toxicity of amrubicin was hematologic. Grade 3 or 4 leukopenia was frequently observed in 51.5% of patients and grade 3 or 4 neutropenia in 84.8% of patients. Despite such severe hematologic toxicity, 88% of the total treatment cycles could be delivered without dose reduction and non-hematologic toxicities were mild. Although anorexia (54.5%) and nausea and vomiting (57.6%) were frequently observed, there were no episodes of grade 3 or 4 toxicity, except for 3 patients (9.1%) with grade 3 anorexia and 1 patient (3.0%) with grade 3 alopecia. A single patient developed interstitial pneumonia; however, this was reversible with steroid therapy. ECG abnormalities were observed in 3 patients, but they were each reviewed by a medical cardiologist and judged not to be clinically significant. No LVEF decrease was observed. Results show that the toxic profiles of amrubicin are acceptable and favorable in the treatment of extensive-disease SCLC, although due to its hematologic toxicity, in particular neutropenia, G-CSF support is needed.

In conclusion, amrubicin is a very active and promising agent with acceptable toxicity for patients with SCLC. Further studies are warranted in combination with other agents for this disease.

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Three-dimensional Conformal Radiation Therapy for In Situ or Early Invasive Central Airways Lung Cancer

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Introduction: Central airways lung cancer is occasionally discovered in early stage. Because of comorbidities, surgical resection is not always advisable for this type of lung cancer. Photodynamic therapy or endobronchial brachytherapy can produce cure for centrally located small lung cancers and is an alternative for surgery in selected patients. However, their application is limited by size and depth of invasion of the tumors or bronchoscopic access. External beam radiation can be applicable to almost all patients, when planned well. In this study, we evaluate the safety and efficacy of 3-dimensional conformal radiotherapy (3D-CRT) for in situ or early invasive central airways lung cancers.

Methods: Between November 2001 and December 2004, 8 patients with newly diagnosed or recurrent central airways lung cancer without nodal and distant metastasis were treated by 3D-CRT of 60 Gy in 3-Gy fractions. Target volume included the primary tumor but did not include regional lymph nodes. All patients were evaluated for disease control, survival, and complications.

Results: All lesions responded to the treatment. The median survival time was 36.8 months (30 to 50 mo), and the cause-specific survival time was 36.8 months (30 to 50 mo). Two-year overall, cause-specific survival, and locoregional control rate were 100%. Toxicity included pneumonitis observed in 1 patient, which resolved by conservative therapy.

Conclusions: 3D-CRT is a safe and effective treatment modality for in situ or early invasive central airways lung cancer when surgical resection or endobronchial therapy is not advisable.

Key Words: conformal radiotherapy, in situ or early invasive central airways lung cancer, local control, radical radiotherapy

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Lung cancer is occasionally detected as small centrally located tumors such as carcinoma in situ (CIS) or early invasive cancer. When lymph node and distant metastasis are not present, good clinical outcome including cure can be expected. Surgical resection, photodynamic therapy (PDT), and endobronchial brachytherapy are the modalities of treatment of choice. Although surgical resection is the standard treatment for early invasive central airways lung cancer, elderly patients or those with severe comorbidities are frequently determined medically inoperable. Additionally, as most of CIS or early invasive central airways lung cancers are smoking-related and have a tendency to be multifocal, conservative treatment is often sought. PDT is less invasive and effective for CIS or early invasive cancer, but complete remission is unlikely with large lesions and those deeper than bronchial cartilage.[1] In endobronchial brachytherapy, control of radiation dose is difficult and could lead to massive hemoptysis and exsanguination.[2]

Although external beam radiation remains an option for these patients, conventional one is associated with poor outcomes with 5-year survival rates of 25% to 30%.[3–17] Dose escalation of radiation using conventional fractionation and techniques would likely cause prohibitive toxicity. Three-dimensional conformal radiotherapy (3D-CRT) is intended to deliver higher doses of radiation, while minimizing damage to surrounding normal tissues. Because good results are reported in 3D-CRT for stage I peripheral lung cancer, 3D-CRT may have a potential to be curative for central-type lung cancers.[18] However, high-dose irradiation to hilar regions is still considered to be unsafe.[19] However, high but acceptable dose of irradiation seems to be necessary for centrally located small lung cancers.

Since 2001, we have been treating CIS and early invasive central airways lung cancer using 3D-CRT, when the lesions were inoperable or too invasive to treat with PDT. In this manuscript, we report the safety and efficacy of 3D-CRT for small centrally located lung cancers.

MATERIALS AND METHODS

Patient Characteristics

Between November 2001 and December 2004, 8 centrally located lung cancers without nodal (N0) and

TABLE 1. Patient Characteristics

Number	Age	BI	Localization	Size (mm)	Prior Therapy for Lung	Comorbidity
1	78	1200	Carina-rt., main-rt., second carina	35	Rt. B6 segmentectomy, rt. lower lobectomy	HT, cerebrovascular disease
2	56	1050	Lt. B6/basal bronchus spur-B8+9/10 spur	15	PDT for lt. second carina and lt. B6, lt. B6 segmentectomy, endobronchial brachytherapy for lt. B6	HT, chronic hepatitis
3	74	1320	Lt. upper bronchus	15	No	HT, COPD, hard of hearing
4	64	800	Rt. middle bronchus	25	PDT for rt. middle bronchus	Gastric ulcer, arrhythmia, COPD
5	80	1200	Rt. main	20	No	COPD
6	74	1000	Lt. upper bronchus	15	No	Renal dysfunction
7	67	800	Rt. basal bronchus	15	Lt. Lower lobectomy	No
8	71	1320	Rt. upper bronchus	25	Carina resection and tracheoplasty, Lt. basal segmentectomy	HT

BI indicates Brinkman Smoking Index; COPD, chronic obstructive pulmonary disease; HT, hypertension; Lt., left; PDT, photodynamic therapy; Rt., right; SCC, squamous cell carcinoma.

distant metastasis (M0) in 8 patients were treated with 3D-CRT with curative intent. Central lung cancer is defined as that originated from airways including and proximal to subsegmental bronchi.[20,21] All lesions were cytologically or histologically proved as squamous cell carcinoma and located from carina up to the segmental bronchus. No tumors could be detected by conventional chest computed tomography (CT). The local spread of the lesions was determined by conventional and autofluorescence bronchoscopy, together with endobronchial ultrasonography. Routine staging of the disease included chest x-rays and CT scans of thorax and abdomen. Brain CT/magnetic resonance imaging and bone scintigraphy/positron emission tomography were not mandatory in the cases of CIS.

Pretreatment characteristics of all 8 patients are shown in Table 1. They were all males and smokers/ex-smokers, whose Brinkman smoking indices were ranged between 800 and 1320. The median age was 71 (range: 56 to 80) years. Eastern Cooperative Oncology Group performance status was 0 in all patients. Most patients were considered to be inoperable, mostly as a result of comorbidities and poor pulmonary function owing to previous surgery, higher age, or chronic obstructive pulmonary disease. Two patients (nos. 1 and 8) experienced stump recurrences at the bronchial resection margins. In 1 patient (no. 7), a new primary lesion appeared away from the stump region. Another one (no. 2) was treated by PDT twice, surgery and endobronchial brachytherapy for the left lower lobe endobronchial cancer, yet developed recurrence. Another one (no. 4) had CIS and received prior PDT for the lesion, but complete regression could not be attained. The remaining 3 patients (nos. 3, 5, and 6) were considered to be inoperable mostly as a result of comorbidities and endobronchial therapy, such as PDT or brachytherapy, was not indicated owing to the extent of the lesions. Patient nos. 3 and 5 had CIS. As conformal radiotherapy (CRT) is considered to be the only available curative treatment, the modality was used after obtaining informed consent.

Treatment

Plain CT images of 0.5-mm thickness were obtained over whole lungs. The images were transferred to radiation planning computer (CADPLAN, Varian Medical Systems, Palo Alto, CA) to make 3D-CRT plans. As the tumor could not be depicted on CT images, clinical target volumes (CTVs) were defined as possible tumor length along the bronchial tree and tumor depth into the bronchial wall on the basis of bronchoscopical findings. Hilar, mediastinal, and supraclavicular nodal regions were not included in CTV. The planning target volume (PTV) was designed by enlarging CTV in all directions by 8 to 10 mm, taking both setup uncertainty and respiratory movement into considerations. Radiation fields were formed with multileaf collimator to achieve conformity with leaf margin of 5 mm and coplanner 5-beams arrangement. Beam energy was 6 or 10 MV x-ray. Figure 1 shows an example of 3D-CRT planning for a central-type lung cancer.

Total 60 Gy, prescribed at the isocenter, was administered by 3-Gy fraction, once a day for 4 weeks. V_{20} of the lungs was defined as the percentage of lung volume that received ≥ 20 Gy radiations in the treatment plan. The biologic effective dose (BED) was calculated using the following formula: $BED = nd\ [1 + d/(\alpha/\beta)]$ where n = number of fractions, d = fraction dose, and α/β is assumed to be 10 for tumor cells or acute responding tissues.

Tumor response was evaluated by bronchoscopy and chest CT. Chest x-ray and CT were examined regularly. Radiation-induced toxicities were graded according to the Radiation Therapy Oncology Group/European Organization for Research and Treatment of Cancer (RTOG/EORTC) Late Radiation Morbidity Scoring Scheme. Pulmonary function tests including percent vital capacity and percent forced expiratory volume in 1 second and arterial blood gas analysis were obtained before and after the treatment to identify the risk factors for lung toxicity by 3D-CRT. Paired t test was used to compare respiratory function and PaO_2 values.

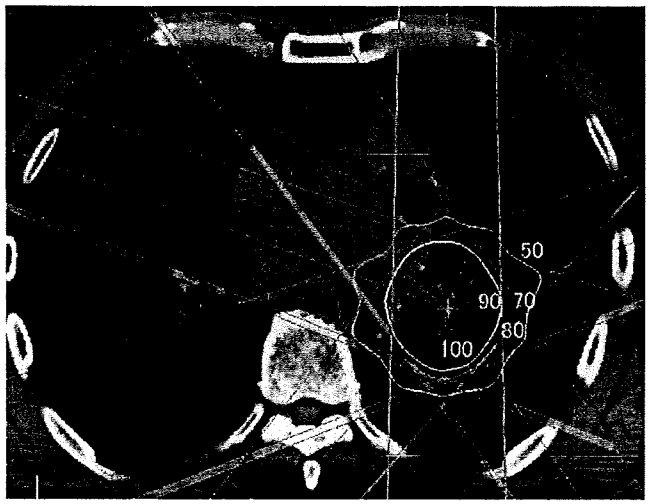

FIGURE 1. The 3-dimensional conformal plan beam arrangement. Circle lines represent the 50% to 100% isodose curves.

RESULTS

The planned treatment was safely performed in all 8 patients with no or minimal acute adverse events. No acute esophageal toxicity was observed. Grade 1 acute radiation pneumonitis (RTOG) was observed in 1 patient. Local response was evaluated by both bronchoscopy and chest CT in 6 patients, but the other 2 patients were considered unsuitable for bronchoscopy and their response was evaluated by sputum cytology and chest CT.

The median follow-up period was 36.8 months (range: 30 to 50 mo). Median survival time was 36.8 months (range: 30 to 50 mo). The 2-year locoregional control rate was 100%. Six patients were alive and 2 died of intercurrent disease without recurrence of centrally located lung cancer. Local failure did not occur in any patient. During follow-up period, secondary lung cancer (adenocarcinoma in both patients) was developed in 2 of 8 patients and they underwent additional 3D-CRT. One of them died of secondary lung cancer due to primary failure at 31 and 10 months after the first and second CRT, respectively. The other patient is alive in the presence of metastasis to the bone and brain, whereas 2 primary sites were maintained to be well controlled in all examinations, including positron emission tomography.

No patient experienced late toxicities at 90 days from the first day of radiation therapy. Table 2 depicts the PTV and the V_{20} values. The median PTV was 45.5 mL (range: 27.6 to 61.8mL) and the median V_{20} value was 10.7% (range: 8.3 to 17.0). We did not encounter interstitial changes in the irradiated lung field with this focal radiation therapy in any of our patients (Figs. 2A, B). Bronchoscopically, the irradiated bronchus was slightly stenotic and scarred (Figs. 3A, B). Respiratory functions and arterial blood gas analysis were unaffected in all patients who underwent the evaluation (Figs. 4A–C). Some patients did experience acute radiation esophagitis, yet it was in grade 2 or less at each occasion.

DISCUSSION

Natural history of CIS and severe dysplasia in the respiratory tract is not clarified completely, and therefore, their treatment strategy is still controversial. Although all of these lesions do not necessarily progress to clinically relevant lung cancers,[22] appreciable proportions of them have high risk of becoming invasive carcinoma. Their risk to progress to a clinical lung cancer was reported to be 33% at 1 year and 54% at 2 years.[23] Therefore, these lesions should be treated in their early stages.

Surgery is the standard treatment for early invasive central airways lung cancer in the patients with good performance status. In Japan, 5-year survival rates of the patients with lung cancer treated surgically are 72% for cIA and 49.9% for cIB and 79.5% for pIA and 60.1% for pIB.[24] On the other hand, Kato et al[1] reported that PDT yielded an initial complete response rate of 84.8% for centrally located early-stage lung cancer. PDT is considered as an effective alternative for surgery for centrally located stage 0 (TisN0M0) and stage I (T1N0M0) early invasive lung cancer, when surgical intervention is difficult or the patients refuse surgery. PDT is especially attractive for elderly patients or those in poor physical condition. Whereas PDT is reported to be effective only for the superficial tumors of < 1 cm in diameter with visible peripheral margin and which is located no more peripherally than subsegmental bronchi, another modality is necessary for the tumors that do not fulfill at least 1 of these conditions.

For many years, the mainstay of treatment for inoperable lung cancer was radiation of nearly 60 Gy of total dose with 2 Gy/fraction over 6 weeks. Conventional external beam radiation of 60 to 70 Gy alone is reported to result in 15% of 5-year overall survival rate, 25% intercurrent death rate, and 50% of treatment failures in local site alone, in the expense of grade 3 to 5 complications of $< 5\%$.[25] These results are not satisfactory for stage I lung cancer. On the basis of dose-response data, Mehta et al[26] estimated that it would take a dose of approximately 85 Gy to achieve 50% long-term control rate using standard 2-Gy daily fractions. It seems that higher doses and shorter treatment times are required to achieve better disease control. However, radiation dose escalation using conventional fractionation and

TABLE 2. Planning Target Volume and V_{20}

Number	PTV (mL)	V_{20} (%)
1	58.24	8.50
2	36.30	9.80
3	36.00	11.78
4	61.80	11.42
5	47.40	9.14
6	36.32	9.35
7	27.60	8.30
8	60.02	16.95

V_{20} was defined as the percentage of lung volume that received ≥ 20 Gy radiations in the treatment plan.
PTV indicates planning target volume.

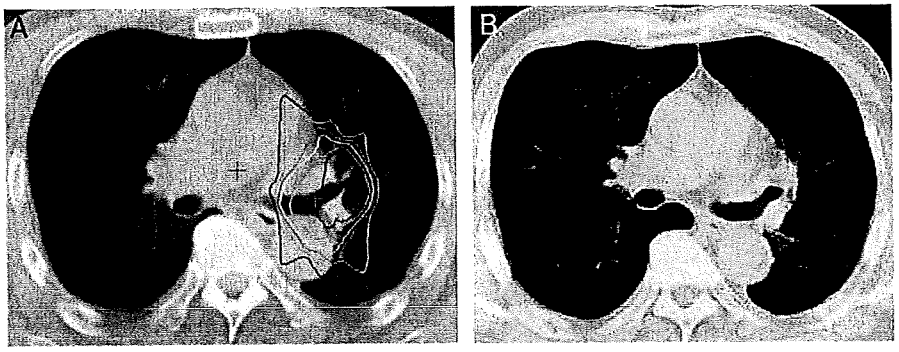

FIGURE 2. A, An example of the dose coverage on an axial CT image in the 74-year-old patient with cancer located at left upper bronchus. B, CT scan at 1-year follow-up shows a complete response without post-treatment interstitial lung changes. CT indicates computed tomography.

techniques would likely cause prohibitive toxicity. 3D-CRT is intended to deliver higher dose of radiation while minimizing damage to surrounding normal tissues. We treated the patients by CRT with 20 fractions of 3 Gy. The biologically effective dose (BED) of this radiation is calculated to be almost equal to 78 Gy in conventional fractionation (assuming α/β of 10). Almost no, at most minimal, interstitial changes were observed in the irradiated lung fields (Figs. 2A, B). This observation was further supported by the fact that respiratory functions were unaffected by the treatment in all patients. These are ascribed to very limited PTV with a median of 45.5 mL. Lagerwaard et al[27] showed that central location of tumors (endobronchial tumor extension) was the only factor that significantly reduced local progression-free survival in 3D-CRT for lung cancer. Our good results can be ascribed to small size of the tumors, which do not require large dose of radiation compared with established invasive cancer. Recently, stereotactic radiotherapy (SRT) is showing favorable results in the treatment of peripherally located stage I lung cancer. Timmerman et al[19] reported a phase 2 trial of SRT with 60 to 66 Gy in 3 fractions during 1 to 2 weeks in 70 patients with medically inoperable early-stage lung cancer. Grade 3 to 5 toxicity occurred in 14 patients (20%). In 2-year follow-up after SRT, 83% of the patients with peripherally located lung cancer experienced no severe complications, whereas 54% of those with centrally located cancer did. The patients with centrally located tumors have 11-fold increased risk of experiencing severe complications compared with those with lung cancer located more peripherally. Their conclusion was that SRT of this regimen should not be used for the patients with tumors located near the central airways because of excessive complications. Similarly, Le et al[28] reported the results of dose-escalation study using single-fraction SRT of 15 to 30 Gy for lung tumors. Majority of the patients who showed grade 2 or greater complications had either centrally located tumors and/or the tumors with treatment volumes greater than 50 mL. The toxicities observed included pneumonitis, pleural effusion, pulmonary embolism, and tracheoesophageal fistula. These results indicate that high-dose radiation by limited fractions is dangerous for perihilar structure of the lung. As small lung cancer,

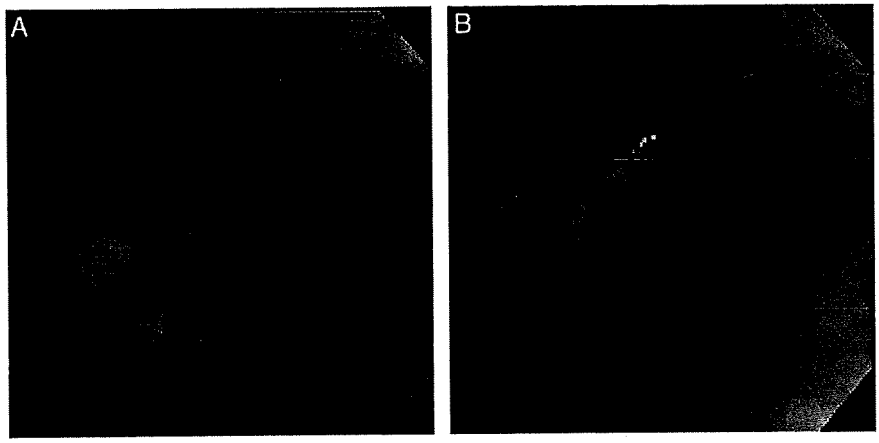

FIGURE 3. A case of 74-year-old patient with central type lung cancer. A, Squamous cell carcinoma located at left upper bronchus. B, After 6 months of 3D-CRT, the irradiated bronchus was slightly stenotic and scarred. 3D-CRT indicates 3-dimensional conformal radiotherapy.

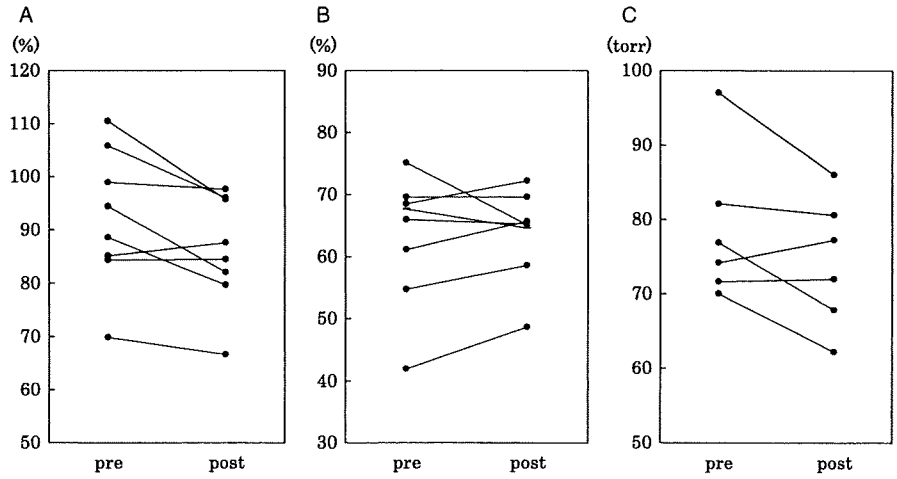

FIGURE 4. Respiratory function values of preradiotherapy and postradiotherapy. A, %VC: percent vital capacity. B, FEV1.0%: percent forced expiratory volume in 1 second. C, PaO_2: arterial blood gas analysis.

such as CIS and early invasive cancer, is curative by radiation with sufficient dose, determination of total dose and fractionation is critical to treat small lung cancer located in the central airways. Although the number of the patients entered into this study is small, our method may afford a good clue.

CONCLUSIONS

As small lung cancer, such as CIS and early invasive cancer, is curative by radiation with sufficient dose, determination of total dose and fractionation is critical to treat small lung cancer located in the central airway. 3D-CRT given by 20 fractions of 3 Gy is a safe and effective treatment for inoperable CIS or early invasive central airways lung cancer.

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