Adjuvant Chemotherapy Based on the In Vitro Histoculture Drug Response Assay for Non-small Cell Lung Cancer Improves Survival

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**Background:** In this study, we analyzed the usefulness of adjuvant chemotherapy for non-small cell lung cancer based on the histoculture drug response assay (HDRA).

**Methods:** From September 2001 to December 2008, 65 patients with pathologic stage II or higher non-small cell lung cancer who underwent surgery received two-cycle HDRA-based adjuvant chemotherapy. Chemosensitivity to cisplatin, carboplatin, paclitaxel, docetaxel, gemcitabine, and irinotecan was examined by the HDRA. All patients were classified according to the number of administered HDRA-positive drugs: the prediction-sensitive group (PSG) \((n = 31)\) comprised patients treated with two HDRA-positive drugs and the prediction-nonsensitive group (PNSG) \((n = 34)\) comprised those treated with a combination of one HDRA-positive and one HDRA-negative drug or two HDRA-negative drugs. The clinical outcomes of the two groups were analyzed.

**Results:** The overall 5-year survival rate of the PSG was 82.4%. On the other hand, that of the PNSG was 40.1%. There were significant differences between the two groups \((p = 0.03)\). The 5-year disease-free survival rate was more favorable in the PSG than in the PNSG (PSG: 56.5%, PNSG: 30.1%, \(p = 0.05\)). Multivariate analysis showed that chemotherapy based on the HDRA was a significant prognostic factor \((p = 0.03)\).

**Conclusions:** The prognosis of patients treated with two HDRA-positive drugs was significantly better than that of those treated with one HDRA-positive drug or HDRA-negative drugs. Adjuvant chemotherapy based on the in vitro HDRA may be useful to improve survival in patients who have undergone surgery.

**Key Words:** Histoculture Drug Response Assay, Adjuvant chemotherapy, Non-small cell lung cancer. (J Thorac Oncol. 2010;5: 1376–1381)

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Lung cancer is the leading cause of cancer death in many countries. Surgery is the best potentially curative treatment modality for patients with non-small cell lung cancer (NSCLC). NSCLC accounts for about 80 to 85% of all lung cancer. Although the discovery rate of early-stage lung cancer has increased due to the spread of computed tomography-based checkup, lung cancer discovered at an advanced stage is still common. Moreover, it has been reported that the greater part of relapse in patients who have undergone complete resection involves distant metastasis, so it is insufficient solely in local treatment such as surgery. To improve the postoperative survival of patients with NSCLC, the development of effective postoperative therapy is essential. Large numbers of clinical trials involving multimodality therapy are based on such a background, and many trials showing the usefulness of adjuvant chemotherapy have recently been reported.1–6 The International Adjuvant Lung Cancer Collaborative Group Trial demonstrated a 4.1% improvement in survival in patients with stages I to III NSCLC.1 The JBR10 trial demonstrated a 15% improvement in 5-year survival in an adjuvant chemotherapy arm involving stage IB or II patients.2 The Adjuvant Navelbine International Trialist Association trial reported that the overall survival at 5 years improved by 8.6% in the chemotherapy arm and that this survival rate was maintained at 7 years (8.4%) in stage II and IIIA patients.3 Lung Adjuvant Cisplatin Evaluation, a pooled analysis of five randomized trials that included 4584 patients, showed a significant overall survival advantage for all patients with stage II or III cancer who received cisplatin (CDDP)-based adjuvant chemotherapy.4 However, no definite regimens of adjuvant chemotherapy for NSCLC have been determined. Administering inappropriate anticancer drugs has adverse effects on patients. Recently, an in vitro drug response assay, developed over more than four decades, was introduced to individualize chemotherapy as an alternative to empiric therapy. Theoretically, use of the in vitro drug response assay could lead to more rational treatment decisions. Freeman and Hoffman7 introduced a developmental methodology called collagen sponge-gel-supported histoculture, which allows diverse human tumors obtained directly from surgery to grow at a high frequency in vitro for long periods and established the histoculture drug response assay (HDRA). Several researchers in the fields of gastric and esophageal cancer have reported that HDRA-based adjuvant
chemotherapy has the potential to improve patient survival\textsuperscript{8–10}; however, to the best of our knowledge, no work has been done to elucidate the clinical usefulness of the HDRA for NSCLC in an adjuvant setting. So, we retrospectively investigated the usefulness of adjuvant chemotherapy based on the HDRA for patients with NSCLC.

\textbf{PATIENTS AND METHODS}

\textbf{Patients}

From September 2001 to December 2008, 832 patients underwent surgery with curative intent for primary NSCLC in our institute. Of these, tissue samples of primary tumors were collected at surgery from 482. Of the 482 patients, 68 could not undergo sufficient HDRA measurement because of insufficient cell viability or tissue amounts. The remaining 414 patients (85.8\%) could be evaluated regarding their chemosensitivity to CDDP, carboplatin (CBDCA), irinotecan (SN38), paclitaxel (PTX), docetaxel (DOC), and gemcitabine (GEM). A mean of 4.8 ± 1.5 (min–max: 1–6) drugs were tested in the 414 patients. The patients with pathologic stage II or more advanced disease underwent two-cycle adjuvant chemotherapy. If patients showed two or more HDRA-positive drugs, we selected the two strongest HDRA-positive drugs while excluding combinations of the same category (CDDP and CBDCA, PTX, and DOC). If patients showed only one HDRA-positive drug, we selected this drug and the strongest HDRA-negative drug. If patients showed no HDRA-positive drug, we selected the strongest platinum agent and the most powerful drug among nonplatinum agents under informed consent. If a patient showed a poor postoperative performance status (World Health Organization performance status 2 or higher), advanced age (more than 75 years), or severe complications, we performed adjuvant chemotherapy with a single agent using HDRA results. Of the 414 patients, 187 showed pathologic stage II or higher. Of the 187 patients, 143 received adjuvant chemotherapy. Of those, 78 patients were excluded from this study because of single-agent or insufficient chemotherapy. So, in this study, we retrospectively reviewed the remaining 65 patients who received two-cycle HDRA-based adjuvant chemotherapy with a doublet regimen. All patients showed World Health Organization performance status 1 or lower and had no history of concurrent malignant disease. No patients received postoperative radiotherapy.

Pathologic staging was performed according the seventh edition of the tumor, node, metastasis classification of the Union Internationale Contre le Cancer for lung cancer.

Our institutional review board for clinical practice approved this study, and written informed consent regarding the use of the HDRA was obtained from all patients before surgery.

\textbf{Histoculture Drug Response Assay}

Tumor tissue was freshly harvested from surgically resected specimens, excluding necrotic or infected portions, washed in Hanks solution, minced into pieces of approximately 10 mg, and then placed on prepared collagen surfaces in 24-well microplates. Plates were incubated for 7 days at 37°C with 5\% CO\textsubscript{2} in Roswell Park Memorial Institute 1640 medium supplemented with 20\% fetal calf serum and anticancer agents. Concentrations of drugs were 20 \(\mu\text{g/mL}\) for CDDP, 25 \(\mu\text{g/mL}\) for CBDCA, 0.2 \(\mu\text{g/mL}\) for irinotecan (SN38), 100 \(\mu\text{g/mL}\) for DOC, 40 \(\mu\text{g/mL}\) for PTX, and 1000 \(\mu\text{g/mL}\) for GEM. After histoculture, 100 \(\mu\text{L}\) of Hanks balanced salt solution containing 0.1 mg/mL of type I collagenase (Sigma) and 100 \(\mu\text{L}\) of 3-(4,5-dimethyl-2-thiazoyl)-2,5-diphenyl-2H-tetrazoliumbromide (MTT) solution dissolved in 5 mg/mL of phosphate buffer solution was added to each culture well and incubated for another 16 hours. After extraction with dimethyl sulfoxide, the absorbance of the solution in each well was read at 540 nm.\textsuperscript{11,12} The absorbance per gram of cultured tumor tissue was calculated from the mean absorbance of tissue from culture wells, and the tumor tissue weight was determined before culture.

The rate of inhibition was calculated using the following formula:

\[
\text{Inhibition rate (\%)} = \left(1 - \frac{\text{Mean absorbance of treated tumor}}{\text{Weight}}\right) \times 100
\]

The HDRA was regarded as applicable when the mean absorbance of extracted formazan at 540 nm of the control tumor was 15 or more per gram. When the inhibition rate of the drug was a negative value, it was regarded as zero, which meant zero chemosensitivity.

The concentration and cutoff inhibition rate of anticancer drugs were determined according to previously reported clinical response rates of each drug when administered as a single agent (Table 1).\textsuperscript{13} The cutoff level was not changed according to the histology. An anticancer drug with an inhibition rate above the cutoff level was classified as an HDRA-positive drug.

\textbf{Statistical Analyses}

Categorical variables were analyzed by means of \(\chi^2\) analysis and the unpaired \(t\) test. Survival was estimated by means of the Kaplan-Meier method, and differences in survival were determined using log-rank analysis. The Cox proportional hazards regression model was used to assess the

\begin{table}[h]
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\caption{Anticancer Drug Concentration and Cutoff Inhibition Rate}
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\textbf{Anticancer Drugs} & \textbf{Concentration (\(\mu\text{g/mL}\))} & \textbf{Cutoff Inhibition Rate (\%)} \\
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CDDP & 20 & 50 \\
CBDCA & 25 & 30 \\
PTX & 40 & 70 \\
DOC & 100 & 40 \\
GEM & 1,000 & 30 \\
CPT-11 (SN38) & 0.2 & 50 \\
\hline
\end{tabular}
\end{table}

\textsuperscript{a}CBDCA, carboplatin; CDDP, cisplatin; DOC, docetaxel; GEM, gemcitabine; PTX, paclitaxel.
results of multivariate analysis of independent prognostic factors, which include the HDRA, age, gender, pathologic T factor, pathologic N factor, pathologic M factor, and operative curability. Zero time was the date of pulmonary resection, and the final end point of overall survival was death attributable to cancer or due to a cause other than cancer. The final end point of disease-free survival was the date of recurrence and that of the last follow-up or the date of death in the absence of recurrence. Not all recurrences were confirmed pathologically. A p value of less than 0.05 was considered significant.

RESULTS

From September 2001 to December 2008, there were 65 patients who received HDRA-based adjuvant chemotherapy with a doublet regimen. The 65 patients were divided into a prediction-sensitive group (PSG) (n = 31) and prediction-nonsensitive (PNSG) group (n = 34) according to the number of administered HDRA-positive drugs. The PSG consisted of patients treated with two HDRA-positive drugs, and the PNSG comprised patients treated with a combination of one HDRA-positive drug and one HDRA-negative drug or two HDRA-negative drugs. The overall follow-up period ranged from 6.5 to 85.1 months, with a median of 29.2 months (mean follow-up: 29.2 ± 21.1 months). Table 2 shows the characteristics of the PSG and PNSG. Overall, 15 (23%) patients had postoperative stage IIA disease, 13 (20%) had stage IIB disease, 28 (43%) had stage IIIA disease, 2 (3%) had stage IIIB disease, and 7 (10%) had stage IV disease. Seven patients with postoperative stage IV underwent incomplete resection (slight dissemination in six and slight malignant effusion in one). No differences in the sex, age, histology, pathologic stage, pathologic T factor, pathologic N factor, pathologic M factor, curability of surgery, or epidermal growth factor receptor gene mutation status were observed between the two groups. Adjuvant chemotherapy regimens of the PSG were 80 mg/m² PTX on days 1, 8, and 15 plus 80 mg/m² CDDP on day 1 in 12; 60 mg/m² PTX on days 1, 8, and 15 plus 60 mg/m² camptothecin-11 on days 1, 8, and 15 in 10; 80 mg/m² PTX on days 1, 8, and 15 plus an area under the curve (AUC) of 5 mg/mL per minute CBDCA on day 1 in 6; and 60 mg/m² DOC on day 1 plus 80 mg/m² CDDP on day 1 in 3. Those of the PNSG were 80 mg/m² PTX on days 1, 8, and 15 plus 80 mg/m² CDDP on day 1 in 8; 60 mg/m² PTX on days 1, 8, and 15 plus 60 mg/m² camptothecin-11 on days 1, 8, and 15 in 8; 80 mg/m² PTX on days 1, 8, and 15 plus an AUC of 5 mg/mL per minute CBDCA on day 1 in 9; 60 mg/m² DOC on day 1 plus 80 mg/m² CDDP on day 1 in 6; 30 mg/m² DOC on days 1 and 15 plus an AUC of 3 mg/mL per minute CBDCA on days 1 and 15 in 2; and 1000 mg/m² GEM on days 1, 8, and 15 plus an AUC of 5 mg/mL per minute CBDCA on day 1 in 1. In both groups, all patients received two-cycle chemotherapy.

The overall 5-year survival rate was 82.4% for the PSG and 40.1% for the PNSG (Figure 1). A significant difference between the two groups was observed (p = 0.03).

During the follow-up period, recurrence occurred in 29.0% (9 of 31 patients) of the PSG (pulmonary metastasis in five, supraclavicular lymph node metastasis in one, kidney metastasis in one, bone metastasis in one, and dissemination in one) and in 55.8% (19 of 34 patients) of the PNSG (pulmonary metastasis in seven, mediastinal lymph node metastasis in five, bone metastasis in three, brain metastasis in two, and dissemination in two). The rate of relapse was lower in the PSG than in the PNSG (p = 0.02). The 5-year disease-free survival rates were 56.5% in the PSG and.

<table>
<thead>
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<th>TABLE 2. Characteristics of Patients with NSCLC Who Underwent Adjuvant Chemotherapy Based on the HDRA</th>
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<tr>
<td>Characteristics</td>
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<tr>
<td>Males/females</td>
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<td>Age (mean ± SD)</td>
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<td>Adenocarcinoma/SqCC/Others</td>
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<td>pStage IIA/IIB/III/IIIB/IV</td>
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<td>pT1a/T1b/T2a/T2b/T3/T4</td>
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<td>pN0/N1/N2</td>
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<td>pM0/M1a/M1b</td>
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<td>Curability: complete/incomplete</td>
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<td>EGFR mutation: positive/negative/unknown</td>
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<td>Adjuvant chemotherapy regimen</td>
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CBDCA, carboplatin; CDDP, cisplatin; CPT-11, irinotecan; DOC, docetaxel; GEM, gemcitabine; HDRA, Histoculture Drug Response Assay; NSCLC, non-small cell lung cancer; PTX, paclitaxel; SqCC, squamous cell carcinoma.

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30.1% in the PNSG (Figure 2). The disease-free survival rate of the PSG was more favorable than that of the PNSG ($p = 0.05$).

Moreover, Table 3 shows the results of multivariate analysis of independent prognostic factors in patients who underwent adjuvant chemotherapy based on the HDRA, demonstrating that patients subjected to adjuvant chemotherapy using two HDRA-positive drugs showed a significantly longer survival than those who underwent adjuvant chemotherapy using one HDRA-positive drug or HDRA-negative drugs ($p = 0.037$).

### DISCUSSION

The findings of this retrospective study showed that adjuvant chemotherapy with two HDRA-positive drugs improved the overall and disease-free survival rates in patients with NSCLC.

We have been measuring the chemosensitivity of lung cancer tissues to various drugs using the HDRA since September 2001 and applied the HDRA results to adjuvant chemotherapy for NSCLC. We examined the sensitivity of surgical specimens to CDDP, CBDCA, SN38, PTX, DOC, and GEM. We did not examine the sensitivity to vinorelbine in this study because its concentration and cutoff level have not been determined. However, we recently started to examine the sensitivity of vinorelbine.

Several studies concerning chemosensitivity tests for lung cancer were previously reported. They used the MTT assay, human tumor clonogenic assay, HDRA, differential staining toxicity assay, adenosine triphosphate (ATP) assay, extreme drug resistance assay, and collagen droplet embedded culture drug sensitivity test (CD-DST). Some studies indicated the clinical usefulness of chemosensitivity tests for lung cancer. Kolek et al. reported that a combination of CBDCA-based neoadjuvant chemotherapy, surgical resection, and adjuvant chemotherapy achieved satisfactory survival rates in stage IIIA NSCLC, especially in patients with the complete resection of the tumor and those given MTT-directed adjuvant treatment and suggested that MTT testing may help optimize adjuvant chemotherapy. Moon et al. investigated correlations between the ATP assay and clinical outcomes after ATP assay-guided platinum-based chemotherapy in unresectable NSCLC. In that study, the positive/negative predictive values were 61.1% and 78.6%, with a predictive accuracy of 68.8%. The platinum-sensitive group showed a more favorable clinical response ($p = 0.036$), longer progression-free survival ($p = 0.060$), and longer overall survival ($p = 0.025$). Kawamura et al. prospectively evaluated the clinical feasibility and efficacy of CD-DST in unresectable NSCLC. CD-DST yielded successful results in 61.3% of patients. Medicare survival times of patients treated with in vitro sensitive regimens were longer than those of patients treated with empirical standard chemotherapy, and the response rate for the in vitro optimal regimen was 72.7%. Yoshimasa et al. examined the chemosensitivities of 359 lung cancer specimens. In this study, the applicability of the HDRA was high at 97.4%, and good predictability (true-positive and true-negative rates of 73.2% and 100%, respectively)
tively, with an accuracy of 83.0%) was observed. In another study, they evaluated the chemosensitivities to gefitinib of NSCLC using the HDRA. A significant relationship between the rate of inhibition and gefitinib concentration was observed (p = 0.016). They concluded that the HDRA could evaluate gefitinib sensitivity in NSCLC. The HDRA has also been used to determine the influence of class III β-tubulin expression in patients with completely resected NSCLC.

We have performed the HDRA to assess 482 fresh surgical specimens, and the assay failed in 68 cases. Therefore, the rate at which the HDRA could be applied for evaluation was 85.8% (414/482). This rate is considered to be higher than that of other assays. This finding is congruent with other studies concerning a variety of cancers, including head and neck, gastrointestinal tract, breast, and urological cancer. The reason for this is that HDRA facilitates a native-state histoculture including tissue architecture, tumor-stromal interaction and differentiated functions, a long-term cell culture, and long-term exposure to time-dependent anticancer drugs. The HDRA offers the advantage of the very high-level preservation of the in vivo tissue architecture observed in vitro compared with other chemosensitivity tests. However, for effectiveness, the HDRA requires a large amount of tissue, and so application is limited. To obtain sufficient specimens for the HDRA, some surgical procedures must be performed even for inoperable cases. In resectable cases, we can obtain a sufficient amount of specimen on the radical resection of lung cancer and use the results of the HDRA for adjuvant chemotherapy. A mean of 1.4 ± 1.2 (min–max: 0–6) HDRA-positive drugs was obtained. Cases showing no positive drugs totaled 25.8% (107/414), and cases showing 2 or more positive drugs totaled 39.6% (164/414). This finding was congruent with a previous study reporting the HDRA in NSCLC.

If we conclude that in vitro chemosensitivity test-guided chemotherapy improves patient survival, we should show that chemosensitivity is not associated in any way with the biologic behavior of cancer cells. So, we divided patients with pathologic stages I to IV NSCLC who had undergone the HDRA but had not received sufficient chemotherapy after the operation into high-sensitive (82 patients who showed two or more HDRA-positive drugs) and low-sensitive (102 patients who showed one or no HDRA-positive drugs) groups and compared the prognoses. There were no significant differences in sex (males/females: 61/21 and 72/30, respectively, p = 0.56), histology (adenocarcinoma/squamous cell carcinoma/others: 51/28/3 and 73/22/7, respectively, p = 0.13), pathologic stage (IA/IB/IIA/IIIB/IIB/IV: 29/31/8/3/7/0/4 and 42/29/6/8/8/9 respectively, p = 0.37), pathologic T-factor (T1a/T1b/T2a/T2b/T3/T4: 13/17/37/8/4/3 and 17/26/37/6/15/1, respectively, p = 0.16), pathologic N-factor (N0/N1/N2: 73/4/5 and 87/6/9, respectively, p = 0.74), and survival rate between the two groups (5-year survival rate: 54.2% and 72.6%, respectively, p = 0.25). This means that sensitivity to anticancer drugs based on the HDRA itself is not a prognostic factor, as described in a previous report on the HDRA in patients with gastric carcinoma6 and the CD-DST in patients with NSCLC.

Because the number of cases in this retrospective study was limited and the follow-up period was short (average period: 29.2 months), a definite conclusion is difficult. However, the prognosis of the PSG was significantly better than that of the PNSG, and so adjuvant chemotherapy based on the HDRA is expected to improve survival. The HDRA seems to be useful for the selection of anticancer drugs in chemotherapy after surgery, although it is necessary to increase the number of cases in the future and extend the period of surveillance. This will be confirmed in a proper randomized control study of the HDRA comparing patient survival on receiving CDDP-containing regimens versus a PSG.

REFERENCES


