Prediction of chemotherapeutic effect on postoperative recurrence by in vitro anticancer drug sensitivity testing in non-small cell lung cancer patients

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A R T I C L E   I N F O
Article history:
Received 22 February 2009
Received in revised form 24 June 2009
Accepted 6 July 2009

Keywords:
Non-small cell lung cancer (NSCLC)
Chemosensitivity test
Collagen gel droplet embedded culture drug test (CD-DST)
Postoperative recurrence
Chemotherapy

A B S T R A C T

Background and aims: The collagen gel droplet embedded culture drug test (CD-DST), is an in vitro anticancer drug sensitivity test. The test has been used with various types of malignant tumors, but the significance of clinical application remains unknown. The aim of the present study is to evaluate the ability of this test to predict the response to chemotherapy in non-small cell lung cancer (NSCLC) patients.

Methods: From January 2000 through March 2007, CD-DST data using the primary tumor specimens to anticancer drugs such as cisplatin (CDDP), carboplatin (CBDCA), paclitaxel (PAC), docetaxel (TXT), gemcitabine (GEM), and vinorelbine (VNR), was successfully obtained from 382 patients that underwent a radical resection for NSCLC. Eighty-one of those patients received 1st line chemotherapy using a “new generation” of anticancer drugs for postoperative recurrence. The chemotherapy regimen consisted of a CDDP (or CBDCA)-based combination (N=41), non-CDDP-based combination (N=1) and single agent (N=39). The predictability of the chemotherapeutic effect by the CD-DST data was analyzed retrospectively.

Results: Partial response (PR) was obtained in 24 patients (response rate = 30%), stable disease (SD) in 33 (41%) and progressive disease (PD) in 24 (30%). Forty-two patients underwent chemotherapy with one or more CD-DST-sensitive drugs, 21 of whom showed PR (RR=50%), whereas only 3 (8%) patients showed PR with chemotherapy with regimen including no CD-DST-sensitive drugs. Good predictability was obtained, with a 50% positive predictive value (PPV) for PR and a 92% negative predictive value (NPV) by CD-DST. The predictive accuracy for the response based on the CD-DST data was 70%. Interestingly, a subset analysis according to recurrence site showed that the predictive accuracy was highest (86%) for CD-DST-based chemotherapy for recurrence in the lymph nodes.

Conclusions: The application of the CD-DST for “new generation” anticancer drugs using surgically resected specimens of primary lesion in NSCLC patients may be clinically useful in the prediction of the response to chemotherapy for postoperative recurrence. CD-DST-oriented chemotherapy for postoperative recurrence especially in the lymph nodes may therefore be promising for the improvement of the treatment outcome.

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1. Introduction

Predicting the chemosensitivity of a patient with malignant disease to an anticancer drug may help to improve the chemotherapeutic response with a possible survival benefit as well as helping to avoid the use of an ineffective anticancer drugs. Chemotherapy based on sensitivity-associated data thus allows the application of individualized therapies [1].

Chemotherapy has been established for non-small cell lung (NSCLC) patients using a “new generation” (or 3rd generation) of anticancer drugs, thus providing a significant improvement in the response rate and the survival benefit. However, such results are still insufficient, and with the development of individualized therapies in various types of malignant tumors, the establishment of a chemotherapeutic regimen based on sensitivity-associated data for NSCLC patients is urgently needed [2].

In vitro anticancer drug sensitivity tests using clinical specimens are representative modalities for providing useful data for designing individualization chemotherapy. Several in vitro anticancer
drug sensitivity tests have been developed in various types of malignant tumors, and such tests have been preliminarily applied experimentally as well as clinically [3,4]. The collagen gel droplet embedded culture drug test (CD-DST) is an in vitro anticancer drug sensitivity test, that has been applied for chemotherapy to NSCLC as well as mesothelioma patients [5–8]. This test uses surgically resected specimens of NSCLC tissues, and provides sensitivity data to anticancer drugs, and has been clinically applied to develop a potential individualized chemotherapy for postoperative recurrence of NSCLC patients [8]. However, the clinical usefulness of this test has not been verified, especially with regard to the “new generation”, or “3rd generation” anticancer drugs.

The aim of the present study was to examine the clinical usefulness of this test in the prediction of response to chemotherapy using “new generation” anticancer drugs to treat postoperative recurrent diseases.

2. Patients and methods

2.1. Patients, tissue specimens, and CD-DST data acquisition

Between January 2000 and March 2007, 1294 patients underwent surgical treatment for lung cancer, and of them, the CD-DST was selectively performed for 435 NSCLC patients using the primary tumor tissue after they provided informed consent. CD-DST was performed as previously described by Kobayashi et al. [5–7]. In brief, each fresh specimen obtained by surgery was minced finely using a scalpel and digested in a cell dispersion enzyme solution (EZ, Nitta Gelatin Inc., Osaka, Japan) for 2 h. The dispersed cancer cells were washed twice and collected by centrifugation at 250 × g for 3 h, filtered through an 80 μm nylon mesh, and then incubated in a collagen gel coated flask (CG-flask, Nitta Gelatin Inc.) in a CO2 incubator at 37 °C for 24 h. Only the viable cells adhering to the collagen gel were collected and suspended in the reconstructed type I collagen solution (Cellmatrix Type CD, Nitta Gelatin Inc.) with the final density being 1 × 105 cells/ml. Three drops of the collagen–cell mixture (30 μl/drop) were placed in each well of a 6-well multiplate and in a 60 mm dish, and allowed to gel at 37 °C in a CO2 incubator for 1 h. The final concentration was about 3 × 103 cells/collagen gel droplet. Culture medium was overlaid on each well and the plate was incubated in a CO2 incubator at 37 °C overnight.

The anticancer drug was added, and incubated for 1 h (gemcitabine) or 24 h (other drugs). After the removal of the medium containing anticancer drugs, each well was rinsed twice, overlaid with serum-free culture medium (PCN-1, Nitta Gelatin Inc.), and incubated for 7 days. On the fourth day of the incubation, the medium was changed once. At the end of incubation, the neutral red was added to each well at a final concentration of 50 μg/ml, and colonies in the collagen gel droplets were stained for 3 h. Collagen droplets in the 60 mm dish were stained just before exposure (day 1). Thereafter, each collagen droplet was fixed with 10% neutral formalin buffer, washed in water, air dried, and quantified by image analysis. The growth rate of control was calculated as the total volume of the control group on day 7/total volume at day 1. When a growth rate was 0.8 or less, the assay was judged as unsuccessful irrespective of the volume of the control group (judged as low growth rate).

The in vitro sensitivity was expressed as the T/C ratio (%), where T was the total volume of the treated group and C was the total volume of the control group. A T/C (%) of 50% or less to each anticancer drug was regarded as in vitro sensitive.

CD-DST data for two drugs or more were successfully obtained in 382 (88%) patients. These data were saved for chemotherapy in the event of postoperative tumor recurrence.

2.2. Anticancer drugs

The anticancer drugs tested in the CD-DST were 0.2 μg/ml cisplatin (CDDP), 2.0 μg/ml carboplatin (CBDCA), 1.0 μg/ml paclitaxel (PAC), 0.1 μg/ml docetaxel (TXT), 0.05 μg/ml vinorelbine (VNR), and 8.0 μg/ml gemcitabine (GEM). The culture time was 1 h only for GEM, while it was 24 h for the other drugs [5–7,9,10].

2.3. Patients receiving chemotherapy for postoperative recurrent disease

Tumors recurred postoperatively in 120 of 382 patients that underwent the CD-DST by November 2008, and consequently a total of 81 patients received chemotherapy as the 1st line regimen. The clinicopathological characteristics of these patients are summarized in Table 1. Forty-nine patients were male, and 32 were female. The histological types were follows: 64 patients had adenocarcinoma, 11 squamous cell carcinoma, and 6 large cell carcinoma. The pathological stage at surgery was IA or IB in 10 patients (pts), IIA or IIB in 20 pts, IIIA or IIIB in 48 pts, and IV in 3 pts (2 pts with pulmonary metastasis, and 1 patient in postoperative status for brain metastasis). These patients received no adjuvant chemotherapy until the tumor recurred. The recurrent site at the time of chemotherapy was as follows: lymph nodes at the local, supravacular, cervical, and/or retroperitoneal sites in 22 patients, lung parenchyma in 16 pts, bone in 14 pts, pleural in 8 pts, other solitary distant sites in 6 pts (liver in 3, adrenal gland in 2, and subcutaneous tissue in 1), and multiple combined sites including lymph nodes, lung, bone, pleura and/or distant organs in 15 patients.

The 1st line chemotherapies for these patients were administered using the following regimens: CDDP- or CBDCA-based combination with some drugs of a new generation for 41 patients (CBDCA + PAC for 18 patients, CDDP + VNR for 10 pts, CBDCA + GEM for 8 pts, and CDDP + GEM for 5 pts), non-platinum combination for one patient (GEM + VNR), and single agent chemotherapy for 39 pts (VNR for 22 pts, TXT for 9 pts, and GEM for 8 pts). These chemotherapy started generally with the standard protocol of the standard doses and cycles of each regimen [11,12].

Table 1
Clinicopathological characteristics of patients. Number of patients, N=81 (N=42).

<table>
<thead>
<tr>
<th>Median age at surgery</th>
<th>Gender male/female</th>
<th>Histology adenocarcinoma</th>
<th>Squamous cell carcinoma</th>
<th>Large cell carcinoma</th>
<th>P-stage at surgery I/II/III</th>
<th>Recurrent site</th>
</tr>
</thead>
<tbody>
<tr>
<td>62 years (range 36–75 years)</td>
<td>49/32</td>
<td>64</td>
<td>11</td>
<td>6</td>
<td>10/20/48/3</td>
<td>Lymph node</td>
</tr>
<tr>
<td>Lung</td>
<td>Bone</td>
<td>Pleura</td>
<td>Others (distant, solitary)</td>
<td>Multiple (systemic)</td>
<td>Regimen combination</td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>16</td>
<td>14</td>
<td>8</td>
<td>6</td>
<td>CBDCA + PAC</td>
<td></td>
</tr>
<tr>
<td>CBDP + VNR</td>
<td>CBDCA + GEM</td>
<td>CDDP + GEM</td>
<td>GEM + VNR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18 (11)</td>
<td>10 (7)</td>
<td>8 (4)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single VNR</td>
<td>TXT</td>
<td>GEM</td>
<td>22 (8)</td>
<td>9 (5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8 (4)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Numbers in parentheses indicate the number of patients undergoing chemotherapy including one or more in vitro sensitive agent based on CD-DST.
The response of recurrent tumor was assessed according to the Response Evaluation Criteria in Solid Tumor (RECIST) criteria [13], generally after two cycle of each combination regimen, and after more than two cycles of each single agent regimen. CR (complete response) was defined as the disappearance of all target lesions for at least 4 weeks. PR (partial response) was defined as a 30% decrease in the sum of the longest diameter of target lesions for at least 4 weeks without the development of new metastatic lesions. PD (progressive disease) was defined as a 20% increase in the sum of the longest diameter of target lesions or the appearance of new lesions. If no response or progression of the disease occurred during the chemotherapy, then the therapeutic effect was judged to be stable disease (SD). The response rate was defined as the total of CR plus PR rate.

2.4. Sensitive regimen and non-sensitive regimen

The sensitive regimen of chemotherapy for the tested patients was defined as that including one or two in vitro sensitive anticancer drug(s) according to the CD-DST data, while the non-sensitive regimen was no in vitro sensitive anticancer drug.

2.5. Statistical analyses

Statistical analyses were performed using Fisher’s exact probability test. The level of significance was set at 5%.

3. Results

3.1. Anticancer drug administration and assessment of response

The 1st line chemotherapy was performed according to the protocol schedule with at least two cycles of the regimen in 74 (91%) of the tested patients. Seven patients received only one cycle of chemotherapy because of some adverse events (in 3 pts) and PD (in 4 pts). Finally, the median number of each chemotherapy cycles was three, ranging 1–10.

Therefore, the response chemotherapeutic response after finishing two cycles of each regimen was assessed only in 74 patients. For the patients (N = 7) receiving only one cycle of chemotherapy, the response was practically assessed at the end of 1st line treatment.

3.2. Sensitive and non-sensitive regimen (Table 1)

Forty-two of the tested patients (52%) received a sensitive regimen of chemotherapy. The number of sensitive regimen to each type of chemotherapy is shown in Table 1.

3.3. Overall response and analysis according to CD-DST results (Table 2)

In the present series, PR was observed in 24 patients (30%), SD in 33 pts (41%), and PD in 24 pts (30%). No CR was obtained, thus the overall response rate was 30%. There was no difference in the response rate between combined and single agent chemotherapy (Table 2, left column).

The response according to the CD-DST results is also shown in Table 2 (right column). Among the patients receiving sensitive regimen, PR was observed in 21 patients (50%), SD in 13 pts (31%), and PD in 8 pts (19%). In contrast, PR was shown only in 3 patients receiving the non-sensitive regimen (8%), SD in 20 pts (51%), and 16 pts (41%). The response rate (50%) of sensitive regimen was significantly higher than that of non-sensitive regimen (8%; p < 0.001). This significance was found regardless of the use of a single agent (p < 0.01) or a combination (p < 0.01). However, among the patients receiving chemotherapy with non-sensitive regimen, the rate of SD (71%) in a combination was higher than that (36%) in a single agent (p < 0.05, Table 2, right column).

Based on the CD-DST data to chemotherapeutic effect, the sensitivity rate was 88%, and the specificity rate was 63%. There was a 50% positive predictive value (PPV) for PR and a 92% negative predictive value (NPV). The predictive accuracy for the chemotherapeutic effect based on the CD-DST data was 70%.

3.4. Analysis according to recurrence site (Table 3)

The response to each regimen was analyzed according to the recurrence site. Among 22 patients with recurrence in the lymph nodes, 15 pts received chemotherapy using the sensitive regimen, of whom 13 pts (87%) showed PR, while 7 pts were treated with the non-sensitive regimen chemotherapy, and only one (14%) of them showed PR. The PPV based on the CD-DST was 87%, and NPV was 86%. The predictive accuracy of the CD-DST data for recurrence in the lymph nodes was the highest (86%). There was a significant difference in the response rate between sensitive and non-sensitive regimens to response (p < 0.01). In contrast, such an association was not observed in other recurrence sites. It was also noted that there was no responder among the patients treated with non-sensitive regimen chemotherapy when tumor recurred in any tissue other than the lung.

4. Discussion

In Japan, as well as in other countries, an in vitro chemosensitivity test for malignant tumors has been developed and introduced clinically. Four tests have been widely applied because they have a
Table 3

Association between chemotherapeutic effect and CD-DST results according to recurrence site.

<table>
<thead>
<tr>
<th>Recurrence site</th>
<th>No. of patients</th>
<th>Node</th>
<th>Lung</th>
<th>Bone</th>
<th>Pleura</th>
<th>Others (solitary)</th>
<th>Multiple</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemotherapy using sensitive regimen</td>
<td>22</td>
<td>16</td>
<td>14</td>
<td>8</td>
<td>6</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>PR</td>
<td>15</td>
<td>11</td>
<td>4</td>
<td>3</td>
<td>3</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>SD + PD</td>
<td>2</td>
<td>5</td>
<td>4</td>
<td>3</td>
<td>2</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Chemotherapy using non-sensitive regimen</td>
<td>15</td>
<td>11</td>
<td>10</td>
<td>5</td>
<td>3</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>PR</td>
<td>1</td>
<td>5</td>
<td>9</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>SD + PD</td>
<td>6</td>
<td>3</td>
<td>10</td>
<td>5</td>
<td>3</td>
<td>9</td>
<td></td>
</tr>
</tbody>
</table>

Sensitive regimen: regimen including one or more in vitro sensitive agent based on CD-DST.
Non-sensitive regimen: Regimen including no in vitro sensitive agent based on CD-DST.

* Sensitive regimen vs. non-sensitive regimen, p < 0.01.

Kawamura et al. [9] recently reported promising data of CD-DST using biopsy samples for treatment of NSCLC patients. Yoshimasu et al. [26] also emphasized that data acquisition based on HDRA using lung cancer samples may be required to realize individualized chemotherapy for lung cancer patients. Therefore, regardless of the specific method, in vitro chemosensitivity tests may provide a predictive indicator of response to chemotherapy using anticancer drugs of an “old generation” as well as a “new generation” for patients with various types of malignant tumors. The present study was the first reporting a good correlation between in vitro chemosensitivity data and response, using “new generation” anticancer drugs for NSCLC patients with postoperative recurrence.

When evaluating the in vitro chemosensitivity data for combined chemotherapy, treatment including at least one in vitro sensitive anticancer drug was regarded as a sensitive regimen, while that including no in vitro sensitive anticancer drug being as non-sensitive regimen. Of course, in single agent chemotherapy, in vitro sensitivity data could be easily evaluated with clinical response, but in vitro tests, such as the CD-DST and HDRA for combined agents has not been established because of its technical difficulty. Chemotherapy including two or more in vitro sensitive anticancer drugs showed better response than that including only one in vitro sensitive anticancer drug [8]. Such a trend was also observed in the present series, but it was not significant (data not shown). Interestingly, the rate of SD among the patients receiving combined chemotherapy with a non-sensitive regimen was significantly higher than that among the patients receiving non-sensitive single agent chemotherapy (Table 2). This suggested that even non-sensitive anticancer drugs provided a better response with a combined regimen. In these cases, good response could be clinically obtained by using a combined regimen. Therefore, the value of the present evaluation method may be limited to combined chemotherapy, and this problem should be resolved in the future.

In the present study, a good predictability was observed, thus showing the sensitivity, specificity, PPV, and NPV to be 88%, 63%, 50%, and 92%, respectively. The representative results of in vitro sensitivity tests performed clinically in Japan are summarized in Table 4. In lung cancer or NSCLCs, the sensitivity rate is generally 88–100%, specificity 60–81%, PPV 42–73%, and the NPV was generally high (more than 90%) [8,9,26]. The results in the present study were consistent with the results shown in others [9,25]. The data on other tumors [10,18,22,23] show the sensitivity and NPV rates to generally be high, thus indicating that sensitive data by in vitro tests have a tendency towards making an over-diagnosis in predicting good response, whereas resistant data have a tendency towards making an accurate diagnosis.

The clinical effect and CD-DST data in the treatment for recurrent sites yielded interesting results. The CD-DST data had the strongest correlation with the clinical response for lymph node recurrence.
The predictive accuracy of the CD-DST data for recurrence in the lymph nodes was also the highest. In contrast, a poor association between the CD-DST data and response was observed, in particular, there was no responder among the patients with pleural or bone metastasis in the series, in spite of chemotherapy with a sensitive regimen.

The mechanism associated with different results according to the recurrent site is not known, but there are potential explanations. First, since the CD-DST data in the present study were obtained by testing the primary tumor tissues specimens, not recurrent lesions, there may be differences in the chemosensitivity of primary and recurrent tumor tissues. Second, the chemosensitivity status itself in the primary tumor tissues might be biologically associated with the metastatic pattern. For example, lung cancer having a strong potential for bone metastasis might clinically trend to show less sensitivity to anticancer drugs. Third, a good association could not be obtained because of the use of a pharmacologically ineffective concentration of anticancer drugs, for example, in patients with pleuritis carcinomatosa. Therefore, the recurrent site may be considered in performing chemotherapy based on CD-DST data. When tumor recurs especially in lymph nodes, chemosensitivity data based on CD-DST may be quite effective for prediction of the response.

A recent report [9] showed chemotherapy for NSCLC patients using the best regimen, including sensitive or active anticancer drugs selected actively by in vitro sensitivity tests have been performed, and favorable results were observed not only in the clinical response but also in survival. Similar reports in other cancers have been also published [22,23]. Moreover, postoperative adjuvant chemotherapy, using optimized regimen based on such chemosensitivity tests have been aggressively applied [23,27,28]. Although the present study was not designed to compare CD-DST data and the optimal regimen, the results may support the potential usefulness for planning chemotherapy with the optimal regimen. Therefore, when the CD-DST shows sensitivity to several anticancer drugs, chemotherapy should be performed with the best regimen, and this individualized therapy could elevate the response rate approximately by 30–50%, according to the present data of the PPV for PR. This individualized treatment strategy may be the most hopeful for recurrence in the lymph nodes. On the other hand, when there was no sensitive anticancer drug of the “new generation” agents, chemotherapy with other anticancer drugs and/or other treatment modalities may be considered after a thorough consultation with patients, because the response rate may be <10%.

In summary, the CD-DST for “new generation” medicine using surgically resected specimens of primary NSCLC tissues may be clinically useful in the prediction of response to chemotherapy for postoperative recurrence, especially in patients with postoperative recurrence in the lymph nodes. Chemotherapy with the best regimen based on the CD-DST provides a promising individualized treatment for improving the response rate in NSCLC patients.

5. Conflict of interest

The authors have no potential conflicts of interest and nothing to disclose.

Acknowledgment

The authors thank Ms. Eri Yoneima for her valuable technical assistance in the present study.

References


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