Examination of Factors Influencing the Success Rates of an In Vitro Chemosensitivity Test for Lung Cancer Using Collagen Gel Droplet-Embedded Cultures

Takahara Y*, Kawasaki Y, Kato R, Shinomiya S, Oikawa T and Mizuno S

Department of Respiratory Medicine, Kanazawa Medical University, Japan

*Correspondence: Yutaka Takahara, Department of Respiratory Medicine, Kanazawa Medical University, 1-1 Daigaku, Uchinada-machi, Kahoku-gun, Ishikawa 920-0293, Japan, E-mail: takahara@kanazawa-med.ac.jp

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Abstract

Objective: We evaluated the success rates and factors influencing the success rates of the Collagen Gel Droplet-Embedded Culture Drug Sensitivity Test (CD-DST) for pleural effusate in lung cancer patients with carcinomatous pleurisy.

Materials and methods: Thirty-two patients with lung cancer having carcinomatous pleurisy were enrolled in this study between January 2014 and May 2017. Their pleural effusions were analysed by CD-DST, and the subjects were classified into two groups: successful cases and unsuccessful cases, based on whether the test was able to successfully determine drug sensitivity. We investigated whether the properties of the pleural effusate might influence the success rate of CD-DST. We observed that the CD-DST tended to have good success rates in patients with higher pH, higher percentage of lymphocytes, higher cell counts, and lower percentage of neutrophils in their pleural effusate. The red blood cell count was higher in the effusate of unsuccessful than successful cases.

Conclusion: Our results suggest that the diagnostic success rates of CD-DST for pleural effusion from patients with lung cancer might improve by appropriate sample selection.

Keywords: Chemosensitivity test, CD-DST, Pleural effusion, Lung cancer

Introduction

Chemosensitivity tests for lung cancer have been developed in order to select the most effective drug prior to commencement of treatment. However, surgical interventions are typically required to obtain the specimens to be used in the tests. Thus, while chemosensitivity tests have been performed in some lung cancer cases in which surgically resected specimens were used for the evaluation of adjuvant chemotherapy [1,2,3], the tests are not routinely used in patients with unresectable lung cancer [4,5,6].

The Collagen Gel Droplet-Embedded Culture Drug Sensitivity Test (CD-DST) is an in vitro sensitivity test that was developed in Japan and is the only test patented as a chemosensitivity test in Japan. It is used to evaluate the sensitivity of tumor cells to a given drug by culturing the tumor cells in collagen gel and subsequently exposing them to various anticancer drugs. Compared to conventional tests, the CD-DST is advantageous in that it requires a fewer number of cells, eliminates the effects of contamination by fibroblasts during the cell collection process, and allows evaluation of the physiological concentrations of the drugs. These features enable its use for evaluation of drug sensitivity with fluids such as pleural and pericardial fluids; however, the results generated might not be definitive, depending on the number of cells and type of specimens used [5].
To date, several studies have demonstrated the application of CD-DST using primary tumor tissues [7,8,9]. However, the use of pleural fluid in CD-DST has not been examined in detail. Thus, the successful chemosensitivity determination rate of CD-DST using pleural fluid remains unclear.

While the development of molecular targeted drugs and immunotherapies has advanced the treatment of lung cancer, cytotoxic chemotherapy still plays an important role in the treatment of patients without any genetic abnormalities and whose first line of treatment has failed. However, patients with poor performance status (PS) experience worsening of their systemic condition as they progress through the course of treatment, and might have no choice but to transition to palliative therapy before an effective drug can be administered. In this context, CD-DST is expected to contribute to selecting the most effective cytotoxic chemotherapy. However, current health insurance only covers a single use of CD-DST in one’s lifetime. Therefore, it would be beneficial to increase the success rates of CD-DST for the treatment of lung cancer. In the present study, we examined factors that influence the results and success rates of chemosensitivity analysis by CD-DST using pleural fluid.

Materials and Methods

A total of 32 lung cancer patients who developed carcinomatous pleuritis between June 2014 and March 2017 were included. Patients included 25 men and 7 women with the mean age of 71.8 years. The specific tumor types were adenocarcinoma (n=25), squamous cell carcinoma (n=3), neuroendocrine tumor (n=2), adenosquamous carcinoma (n=1), and unknown (n=1). In this study, 32 cases were retrospectively evaluated.

Methods

CD-DST was performed based on the methods previously described by Kobayashi et al. [10,11] using the Primaster® human primary cancer cell culture kit (Kurabo Industries Ltd., Osaka, Japan).

In the present study, CD-DST was defined as success if tumor cells were successfully cultured and chemosensitivity was obtained.

The test was performed using pleural fluid samples collected from the patients, and the patients were subsequently categorized into those with definite and indefinite CD-DST results to compare the characteristics of pleural fluid that might influence the ability of CD-DST to determine chemosensitivity.

This study was approved by the Institutional Review Board of Kanazawa Medical University (approval number: I209).

Statistical analysis

The two groups were compared using Student’s t-test and the Wilcoxon signed-rank test, and the Chi² test was used to examine the chi-square distribution of contingency tables. P<0.05 was considered statistically significant, and all analyses were performed using GraphPad Prism5 software (GraphPad Software Inc., San Diego, CA, USA).

Results

CD-DST provided definite results in 43.8% (14/32) of patients. When performed prior to chemotherapy, it successfully indicated sensitivity in 46% (11/24) of patients. Similarly, when performed after chemotherapy, it indicated sensitivity in 38% (3/8) of the remaining patients. Table 1 summarizes the characteristics of the patients.

CD-DST was more likely to be successful in pleural fluid with a high pH, high lymphocyte ratio, low neutrophil ratio, and high total white cell count. Compared to successful cases, pleural fluid that generated indefinite results had a high red blood cell count (Figures 1a-e). There was no significant difference between the groups in terms of the levels of lactate dehydrogenase (LDH), carcinoembryonic antigen (CEA), albumin and total protein in pleural fluid (Figures 2f-l), or tumor histological type (Figure 3).

| Table 1: Characteristics of the 32 lung cancer patients. |
|---------------------------------------------------------|
| number of patients                                      | Success | failure |
|---------------------------------------------------------|---------|---------|
| age                                                     | 74.6±8.2| 69.5±7.7|
| gender (male/female)                                    | (11/3)  | (14/4)  |
| Histology (adeno/sqcc/adenosqcc/neuroendocrine/unknown) | (9/2/1/1/1) | (16/1/0/1/0) |
| Examination time (before treatment/after treatment)     | (11/3)  | (13/5)  |
Figure 1: Relationship between the properties of the pleural effusate in successful and failure cases of CD-DST.

Figure 2: Relationship between the properties of the pleural effusate in successful and failure cases of CD-DST.
Discussion

A previous study reported that CD-DST was successful as a chemosensitivity test in 74.0% (54/73) of primary lung cancer patients when pleural fluid was used for the test [11]. CD-DST might fail when the test sample contains a low number of tumor cells or is contaminated with bacteria, or when tumor cells are poorly proliferative. However, there are no studies to date that have examined whether the characteristics of pleural fluid affect the success rates of CD-DST.

In the present study, we demonstrated that CD-DST is more likely to be successful when the pH of pleural fluid is high. Patients with malignant pleural effusion whose pleural fluid has a low pH are known to have advanced diseases and poorer survival outcomes. In addition, pleural fluid in malignant pleural effusion that is persistent for several months can have a pH of less than 7.3 [12]. Thus, pleural fluid with low pH might contain cells with poor viability, such as those that have undergone apoptosis. This might result in a low number of viable cells after preculture, as well as poor proliferation of cells, which consequently might result in unsuccessful CD-DST. Chemosensitivity tests are currently indicated as second-line treatments in patients with recurrent or metastatic tumors or those with failed chemotherapy [13]. However, since the pH of pleural fluid decreases as cancer progresses, the success rate of CD-DST might also decrease with time. Therefore, it should be performed at an early stage when possible.

Compared to successful cases in CD-DST, pleural fluid that generated indefinite results had high red blood cell counts. The presence of an excessive amount of red blood cells in pleural fluid samples might influence the proliferation of tumor cells, as well as recovery of tumor cells prior to the test. Thus, it is important to avoid puncturing blood vessels when obtaining a pleural fluid sample in order to improve the success rate of CD-DST.

Conclusion

Our findings suggest that it is important to perform CD-DST as early as possible after the occurrence of carcinomatous pleuritis, as well as to be cautious when performing thoracentesis and to avoid bacterial contamination in order to improve the success rate of CD-DST using pleural fluid. Future studies with additional cases are needed to confirm our results.
Declarations

Ethics approval and consent to participate

This study was approved by the Institutional Review Board of Kanazawa Medical University (approval number: I209). All patients gave their written informed consent for study participation.

Consent for publication

All the study participants gave their consent for publication of the study findings. A copy of the written consent is available for review by the Editor-in-Chief of this journal.

Availability of data and material

Not applicable

Competing interests

The authors declare that there is no conflict of interest.

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Authors’ contributions

All authors read and approved the final manuscript.

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