Elevation of Serum Thioredoxin in Patients with Gefitinib-induced Interstitial Lung Disease

Keiichiro Sakuma¹,*, Hajime Nakamura¹, Takayuki Nakamura², Yuma Hoshino², Shugo Ueda³, Masatake Ichikawa⁴, Chiharu Tabata⁴, Shiro Fujita¹, Katsuhiro Masago¹, Junji Yodoi³, Michiaki Mishima¹ and Tadashi Mio¹

Abstract

Objective  Interstitial lung disease (ILD) is a severe adverse event of gefitinib therapy. However, the mechanism still remains unclear. The objective of this study was to examine whether or not oxidative stress, one of the common factors in drug-associated ILD, is involved in the pathogenesis of gefitinib-induced ILD.

Patients and Methods  Using an enzyme-linked immunosorbent assay (ELISA), we measured the concentration of serum thioredoxin (Trx), a redox-active protein with antioxidative effects, in 44 patients treated with gefitinib, including three patients who had ILD.

Results  In patients who had gefitinib-induced ILD, serum Trx levels were significantly elevated. They decreased after cessation of gefitinib therapy accompanying clinical improvement of ILD.

Conclusion  It was suggested that oxidative stress may be involved as a part of mechanisms causing or worsening gefitinib-induced ILD.

Key words: gefitinib, interstitial lung disease (ILD), oxidative stress, thioredoxin (Trx), lung cancer

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Introduction

Gefitinib is a molecular-targeted and orally active agent that inhibits epidermal growth factor receptor (EGFR) tyrosine kinase and exhibits antitumor activity for advanced non-small cell lung cancer (NSCLC). Interstitial lung disease (ILD) is a severe side effect of gefitinib therapy. According to a 2004 report by AstraZeneca showing the results of a prospective investigation of the adverse events associated with gefitinib therapy, the incidence of gefitinib-induced ILD was 6.47%, and 38.6% of these cases were fatal. Despite the clinical significance of gefitinib-induced ILD, the mechanism underlying its pathogenesis remains unclear.

We hypothesized that oxidative stress is involved in the pathogenesis of gefitinib-induced ILD because it is one of the common factors that cause drug-associated ILD (1). Thioredoxin (Trx) is a redox-active protein with antioxidative effects, and its expression is enhanced by oxidative stress. While Trx levels in serum or plasma are approximately 20 ng/ml in healthy people, these levels are increased in patients with diseases associated with oxidative stress, such as pancreatic cancer (2), hepatitis C virus infection (3), severe burn injury (4), acquired immunodeficiency syndrome (AIDS) (5), rheumatoid arthritis (6), heart failure (7), exacerbation of asthma (8), and acute respiratory distress syndrome (ARDS) (9). In this study, we measured the concentrations of serum Trx in 44 patients treated with gefitinib, including three patients who had gefitinib-induced ILD. In the patients who had ILD, Trx levels were significantly elevated on the day of detection of pulmonary infiltration and decreased after cessation of gefitinib therapy, accompanying clinical improvement of ILD. This result sug-
gests a possibility that oxidative stress may be involved as a part of mechanisms causing or worsening gefitinib-induced ILD.

### Patients and Methods

#### Patient profile and treatment

This study was performed according to the principles of the Helsinki Declaration. We collected blood samples under informed consent from 44 patients with advanced NSCLC who were treated with gefitinib after December 1, 2003 in Kyoto University Hospital. Patients who had taken gefitinib before this date were excluded. They received one tablet (250 mg) of gefitinib after breakfast every day. All were hospitalized during the first two weeks of the treatment.

#### Diagnosis and treatment of gefitinib-induced ILD

All patients underwent periodic chest X-ray on days 8, 15, 22, and 43, counted from the first day of gefitinib treatment (designated as day 1). They also underwent periodic chest computed tomography (CT) on days 29 and 57. Blood examinations were also performed periodically and serum was collected from the same samples until day 56 or until approximately 10 days after cessation of gefitinib therapy for patients who had ILD. Additional radiographic examinations were performed when the patients complained of symptoms suggesting onset of ILD, such as dyspnea, increased cough, and elevation of fever. For patients who developed pulmonary infiltrates, we obtained more information from detailed clinical courses and practicable additional examinations to decide whether the infiltrates were induced by gefitinib or other diseases such as radiation pneumonitis, infectious diseases, lymphangitis carcinomatosis, and collagen diseases. Bronchoalveolar lavage (BAL) was performed if tolerable to exclude infectious diseases and lymphangitis carcinomatosis. Blood examinations for Pneumocystis jiroveci, cytomegalovirus infection, collagen diseases were performed and tumor markers were also evaluated to exclude exacerbation of lung cancer. Comprehensively, we diagnosed the pulmonary infiltrates as gefitinib-induced ILD if we obtained no evidence of other diseases. The consensus of three physicians specializing in respiratory medicine was necessary for the diagnosis. Gefitinib therapy was discontinued on the day of appearance of pulmonary infiltration. Prednisolone (PSL) was administered if they complained of dyspnea or their arterial oxygen saturation values were much lower than those before the appearance of pulmonary infiltration. For patients with diagnosis of gefitinib-induced ILD, the cessation of gefitinib therapy was continued.

#### Measurement of serum Trx levels

Serum Trx levels were measured by an enzyme-linked immunosorbent assay (ELISA) as previously reported (10). The values were corrected with the hemoglobin concentrations in the same serum samples to exclude the contribution by Trx derived from red blood cells (11).

### Results

#### Elevation of serum thioredoxin in patients with gefitinib-induced ILD

Forty-four patients were entered into this study (Table 1). There were three cases (3/44, 6.8%) of gefitinib-induced ILD (Table 2) and all of their pulmonary infiltrations improved gradually after cessation of gefitinib therapy with (patients A and B) or without (patient C) administration of

<table>
<thead>
<tr>
<th>Table 1. Profile of Patients</th>
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<tr>
<td></td>
</tr>
<tr>
<td>Total</td>
</tr>
<tr>
<td>*Age</td>
</tr>
<tr>
<td>Male / Female</td>
</tr>
<tr>
<td>*Brinkman index</td>
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<tr>
<td>Performance status</td>
</tr>
<tr>
<td>0 - 2</td>
</tr>
<tr>
<td>3 - 4</td>
</tr>
<tr>
<td>Past history of ILD (+ / -)</td>
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</table>

*Ages and Brinkman indexes are shown as mean ± SD.

Temporal changes of serum Trx levels in the patients are shown in Fig. 1A-C. The levels on the day of detection of the infiltration showed significant elevation, compared with those on day 1 (p=0.023, paired t-test). They decreased after cessation of gefitinib therapy. In patients without ILD, serum Trx levels showed no significant elevation in any period during treatment, compared with those on day 1 (Fig. 1D).

Comparison of Trx levels on day 1 between patients with and without ILD

To determine whether serum Trx levels can be a predictive marker of incidence of ILD, we compared serum Trx levels on day 1 between two subgroups with and without ILD. They were statistically comparable (Fig. 2), showing that the serum Trx levels at the beginning of gefitinib therapy are not predictive of the incidence of gefitinib-induced ILD.

Discussion

Previous reports have shown that the serum Trx level is a good indicator of oxidative stress (2-9). The present study suggests one possibility that oxidative stress may be involved in the mechanisms causing or worsening gefitinib-induced ILD. Many anti-cancer agents such as bleomycin, Adriamycin, and cisplatin exhibit their anti-tumor effects at least in part by producing reactive oxygen species (ROS). Trx is expressed in bleomycin-injured pulmonary epithelial cells (12). Trx is induced by oxidative stress and secreted from the cells. Trx may be secreted from lungs injured by gefitinib-induced oxidative stress and that the serum Trx level fluctuates reflecting the activity of ILD. However, it is considered that the main pharmacological effect of gefitinib is not directly related to oxidative stress (13). Further study is needed to clarify the mechanism how oxidative stress is generated in gefitinib-induced ILD.

We showed that the serum Trx level is not predictive of the incidence of ILD. In many patients, the serum Trx levels were high at the beginning of gefitinib treatment. Cellular redox status is involved in the growth promotion of cancer cells (14). The serum Trx levels are elevated in patients with pancreatic cancer (2) or hepatocellular carcinoma (15). Advanced lung cancer may be involved in the high Trx levels.

### Table 2. Profile of Patients who Underwent Gefitinib-Induced ILD

<table>
<thead>
<tr>
<th>Patient</th>
<th>A</th>
<th>B</th>
<th>C</th>
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<tbody>
<tr>
<td>Age</td>
<td>59</td>
<td>68</td>
<td>62</td>
</tr>
<tr>
<td>Sex</td>
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<td>Male</td>
<td>Male</td>
</tr>
<tr>
<td>Histology</td>
<td>NSCLC</td>
<td>Ad</td>
<td>Ad</td>
</tr>
<tr>
<td>*Day of diagnosis of ILD</td>
<td>48</td>
<td>6</td>
<td>23</td>
</tr>
<tr>
<td>Symptom</td>
<td>(-)</td>
<td>dyspnea,</td>
<td>(-)</td>
</tr>
<tr>
<td>Stage</td>
<td>IIIB</td>
<td>IIIB</td>
<td>IV</td>
</tr>
<tr>
<td>Brinkman index</td>
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<td>1600</td>
</tr>
<tr>
<td>Performance status</td>
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<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Past history of ILD</td>
<td>(-)</td>
<td>(-)</td>
<td>(-)</td>
</tr>
<tr>
<td>Past history of collagen disease</td>
<td>(-)</td>
<td>(-)</td>
<td>(-)</td>
</tr>
<tr>
<td>*Former thoracic radiotherapy</td>
<td>(-)</td>
<td>(-)</td>
<td>(-)</td>
</tr>
<tr>
<td>*Former chemotherapy</td>
<td>~day - 23</td>
<td>~day - 26</td>
<td>~day - 135</td>
</tr>
</tbody>
</table>

*The days when each patient started to take gefitinib are shown as day 1.

Figure 1. (A-C) Temporal changes of serum Trx levels in three patients who had gefitinib-induced ILD. The day of cessation of gefitinib treatment is shown by an arrow. Comparison between the level on day 1 and that on the day of cessation (*) shows significant difference (p=0.023, paired t-test) (D) Temporal changes of serum Trx levels in patients treated with gefitinib without ILD. Mean levels±one standard deviation on day 1 and following eight periods are shown. Compared with the levels on day 1, the other levels in each of the eight periods are not significantly different (paired t-test). Trx: thioredoxin

Figure 2. Serum Trx levels in patients with and without gefitinib-induced ILD on day 1. (p=0.43, Mann-Whitney test). ILD: interstitial lung disease, Trx: thioredoxin

However, the elevation of the serum Trx levels in patients with gefitinib-induced ILD is not associated with cancer progression, because the levels decreased after cessation of gefitinib therapy.

Endogenous Trx exerts cytoprotective effects against oxidative stress. Trx-transgenic mice are more resistant to bleomycin-induced ILD (16), viral pneumonia by influenza virus infection (17), and brain injury during brain ischemia/reperfusion (18). Intratracheal instillation of diesel exhaust particles (DEP) induced the generation of ROS in the lung, which was attenuated in Trx-transgenic mice (19). Some extracellular Trx enters cells and also inhibits ROS production.
(20). Recombinant Trx protein exerts anti-oxidant effects against DEP-induced oxidative stress in A549 culture cells (19). Intravenous injection of recombinant Trx suppresses the infiltration of leukocyte into the inflammatory site (21). Trx administration attenuates bleomycin-induced ILD and inflammatory cytokine-induced interstitial pneumonia (16). Therefore, curative or prophylactic administration of Trx against gefitinib-induced ILD may be speculated, if involvement of oxidative stress is further elucidated.

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References


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