Tacrolimus and Steroid Treatment for Acute Exacerbation of Idiopathic Pulmonary Fibrosis

Nobuyuki Horita, Makiko Akahane, Yukinori Okada, Yosuke Kobayashi, Takahiko Arai, Izuki Amano, Tomoko Takezawa, Masako To and Yasuo To

Abstract

Objective  Acute exacerbation of idiopathic pulmonary fibrosis (AE-IPF) occurs during the chronic progressive course of idiopathic pulmonary fibrosis. Mortality is estimated to be >70%, because no effective treatment has been established. We evaluated the effectiveness of combination therapy of tacrolimus and methylprednisolone for AE-IPF.

Methods  Patients of AE-IPF treated with methylprednisolone pulse therapy with or without tacrolimus (targeting 20 ng/mL) during the period between January 2001 and April 2010 were retrospectively reviewed. The primary endpoints were survival rate and duration. We also observed lactate dehydrogenase levels, partial pressure of arterial oxygen/fraction of inspired oxygen ratio (P/F ratio), KL-6, occurrence of re-exacerbation, and computed tomography score.

Results  Fifteen Japanese patients [tacrolimus group aged 74.2±6.0 years old (n=5), non-tacrolimus group aged 75.1±12.8 years old (n=10)] were identified. Pre-treatment clinical parameters were not significantly different between the two groups. Four of 5 tacrolimus group patients and 1 of 10 non-tacrolimus group patients survived (p<0.05). The median survival durations were >92 days (tacrolimus group) and 38 days (non-tacrolimus group) (p<0.05). Lactate dehydrogenase levels and the P/F ratio were also significantly favorable in the tacrolimus group. KL-6 and CT score were not significantly different in both groups. Four re-acute exacerbations were observed only in the non-tacrolimus group.

Conclusion  Combined tacrolimus and methylprednisolone pulse therapy mitigates AE-IPF, prevents re-acute exacerbation, and contributes to a better prognosis.

Key words: acute exacerbation of idiopathic pulmonary fibrosis, acute lung injury, acute respiratory distress syndrome, corticosteroid, tacrolimus

(DOI: 10.2169/internalmedicine.50.4327)

Introduction

Idiopathic pulmonary fibrosis (IPF) is a chronic progressive fatal fibrotic lung disease without an identifiable etiology, which is accompanied by the histological pattern of usual interstitial pneumonia (1). During the course of the illness, some patients experience deterioration, which is called acute exacerbation (AE-IPF), characterized by rapidly progressive dyspnea, newly developing opacities on computed tomography (CT) images, and a pathological finding of diffuse alveolar damage pattern (2, 3). AE-IPF is a major concern, because the respiratory condition of patients with this condition often deteriorates suddenly, and the mortality rate is usually reported to be >70% (2, 4-17).

Although immunosuppressive therapy with cyclosporin A (CyA) (7, 10) and polymyxin B-immobilized fiber column hemoperfusion treatment (18) has been reported to be effective to some extent, a sufficiently effective therapy has not yet been established. Some investigators even reported the sterility of intensive care unit and a ventilator for patients with AE-IPF, because the mortality of these patients approaches 100% (9, 11, 16, 19). Novel effective therapy for AE-IPF was anticipated to improve the survival rate.
Tacrolimus selectively inhibits the transcription of interleukin-2 and several other cytokines mainly in T helper lymphocytes by inhibiting calcineurin (20). This medication is often used to avoid rejection after organ transplantation and to control collagen vascular diseases. CyA is also known to have a mechanism similar to tacrolimus, however, the in vitro activity of tacrolimus is 100 times more powerful than that of CyA (21).

In the current report, the initial therapeutic serum tacrolimus level was 20 ng/mL, approximately equivalent to 2000 ng/mL of CyA. Thus, the tacrolimus level reported here was much higher than the attempted CyA blood trough levels (100-150 ng/mL) in the previous report (7, 21). Furthermore, the overall side effect of tacrolimus is the same or even less severe than that of CyA (22).

Tacrolimus treatment has been reported to be effective for interstitial pneumonia related to collagen vascular diseases (23), but no existing report mentions tacrolimus treatment for AE-IPF. In the current study, we report our experience treating 5 AE-IPF patients with tacrolimus and compare the outcome with that in treating 10 patients with non-tacrolimus therapy.

**Methods and Materials**

**Patients**

We reviewed medical records of consecutive patients with AE-IPF treated with methylprednisolone (mPSL) pulse therapy along with or without tacrolimus at the Fraternity Memorial Hospital, a teaching hospital in Tokyo, Japan for the period between January 2001 and April 2010. AE-IPF was diagnosed on the basis of the following criteria (3): (i) Previous or concurrent diagnosis of idiopathic pulmonary fibrosis. If the diagnosis of idiopathic pulmonary fibrosis is not previously established, this criterion can be met by the presence of radiologic and/or histopathologic changes consistent with usual interstitial pneumonia pattern on the current evaluation: (ii) Unexplained worsening or development of dyspnea within 30 days; (iii) High-resolution CT with new evidence of radiologic and/or histopathologic changes consistent with usual interstitial pneumonia pattern; (iv) No evidence of pulmonary infection by endotracheal aspirate or bronchoalveolar lavage; (v) Exclusion of alternative causes, including left heart failure, pulmonary embolism, and identifiable cause of acute lung injury. Other known causes of interstitial lung disease including drug toxicity, environmental exposure, and connective tissue diseases were also eliminated before diagnosing idiopathic pulmonary fibrosis (3).

To exclude them, history, physical exam, echocardiogram, CT scan, aspirated sputum culture, brain natriuretic peptide, anti-nuclear antibody, anti-neutrophil cytoplasmic antibody, rheumatoid factor, and beta-D-glucan were taken. All of the patients in the tacrolimus group received an explanation of the benefit and possible risks of this treatment, and gave written informed consent.

**Treatment**

Patients in the tacrolimus group received slow-tapering mPSL pulse therapy (1000 mg/day for 3 days, 500 mg/day for 2 days, 250 mg/day for 2 days, 125 mg/day for 2 days, and 80 mg/day for 2 days), followed by oral prednisolone (1 mg weight-kg⁻¹/day, reduced by about 20% each week). Patients received continuous infusions of tacrolimus (target blood level, 20 ng/mL was determined based on reports concerning organ transplantation) for 5-14 days, followed by oral tacrolimus (target blood level was 5 ng/mL). Survivors continued on a regimen of oral tacrolimus after discharge. Patients in the non-tacrolimus group received mPSL pulse therapy (1000 mg/day for 3 days) followed by intravenous or oral glucocorticoid (1 mg weight-kg⁻¹/day, reduced by about 10 or 20% each week). None of both groups had polymyxin B-immobilized fiber column hemoperfusion treatment or sivelestat sodium hydrate. This study was approved by the Institutional Review Board in the Fraternity Memorial Hospital.

Supportive care measures for patients in both groups included broad-spectrum antibiotics, total parenteral nutrition, sulfamethoxazole-trimethoprim for the prevention of *Pneumocystis carinii* pneumonia, alendronate for osteoporosis prevention, proton pump inhibitors for gastric ulcer prevention, single-room isolation for prevention of infection, and invasive or non-invasive mechanical ventilation.

**Evaluation of treatment response**

The primary endpoints of this study were (i) survival rate, defined as the rate of patients being discharged alive from the hospital and (ii) duration of survival, defined as the duration from the first day of mPSL pulse therapy. Secondary endpoints were lactate dehydrogenase (LDH) levels (normal range 114-265 IU/L), partial pressure of arterial oxygen/fraction of inspired oxygen ratio (P/F ratio), KL-6, occurrence of re-acute exacerbation, and chest CT score (range: 0-100. Higher score meaning severe IPF) (24).

**Statistical analysis**

All statistical analyses were performed using GraphPad PRISM version 5.02 (GraphPad Software, San Diego, CA). Survival curves were obtained using the Kaplan-Meier method, and survival curves were compared using the log-rank test. Differences between the tacrolimus and non-tacrolimus groups were analyzed using the Fisher’s exact test, the Mann-Whitney test, or analysis of variance. All statistical tests were two-sided, and a p value of less than 0.05 was considered statistically significant (α value=0.05).

**Results**

**Baseline patient characteristics**

Fifteen Japanese patients [(tacrolimus group aged 74.2±
Table 1. Profile of Patients on the First Day of mPSL Pulse

<table>
<thead>
<tr>
<th></th>
<th>Tacrolimus group</th>
<th>Non–Tacrolimus group</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>5</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>74.2 ± 6.0</td>
<td>75.1 ± 12.8</td>
<td>NS</td>
</tr>
<tr>
<td>IPF severity classification</td>
<td>2/2/0/1</td>
<td>6/1/0/2</td>
<td>NS</td>
</tr>
<tr>
<td>Sex (male/female)</td>
<td>2/3</td>
<td>4/6</td>
<td>NS</td>
</tr>
<tr>
<td>P/F ratio</td>
<td>175 ± 77</td>
<td>169 ± 99</td>
<td>NS</td>
</tr>
<tr>
<td>LDH (IU/L)</td>
<td>419 ± 166</td>
<td>682 ± 353</td>
<td>NS</td>
</tr>
<tr>
<td>Duration since diagnosis of IPF (month)</td>
<td>22.4 ± 23.0</td>
<td>26.5 ± 60.0</td>
<td>NS</td>
</tr>
<tr>
<td>Previous diagnosis/ Concurrent diagnosis</td>
<td>4/1</td>
<td>5/5</td>
<td></td>
</tr>
<tr>
<td>Smoking history (CS/ES/NvS)</td>
<td>0/2/3</td>
<td>1/2/7</td>
<td>NS</td>
</tr>
<tr>
<td>Duration between onset and day 0 (day)</td>
<td>10.4 ± 6.3</td>
<td>8.3 ± 7.3</td>
<td>NS</td>
</tr>
<tr>
<td>C-reactive protein (mg/dL)</td>
<td>15.0 ± 10.3</td>
<td>9.3 ± 5.9</td>
<td>NS</td>
</tr>
<tr>
<td>KL–6 (U/mL)</td>
<td>1461 ± 717</td>
<td>1827 ± 1582</td>
<td>NS</td>
</tr>
<tr>
<td>SP-D (ng/mL)</td>
<td>236 ± 107</td>
<td>250 ± 151</td>
<td>NS</td>
</tr>
<tr>
<td>SP-A (ng/mL)</td>
<td>120 ± 103</td>
<td>118 ± 64</td>
<td>NS</td>
</tr>
<tr>
<td>pH on blood gas analysis</td>
<td>7.40 ± 0.07</td>
<td>7.44 ± 0.05</td>
<td>NS</td>
</tr>
<tr>
<td>CT score</td>
<td>29.0 ± 7.3</td>
<td>27.0 ± 6.3</td>
<td>NS</td>
</tr>
<tr>
<td>CT pattern</td>
<td>0/2/3</td>
<td>1/3/6</td>
<td>NS</td>
</tr>
</tbody>
</table>

NS = Not significant; CS = current smoker; ES = Ex–smoker; NvS = never smoker.

The difference between two groups was analyzed using the Fisher’s test, the Mann–Whitney test, or analysis of variance.

Mean ± Standard deviation

6.0 years old (n=5) and non-tacrolimus group aged 75.1±12.8 years old (n=10), were evaluated. All fifteen patients satisfied five diagnostic criteria. Open lung biopsy was not done for any patients. Transbronchial lung biopsy specimen was obtained for one patient in the tacrolimus group and one patient in the non-tacrolimus group, which was compatible with the diagnosis of AE-IPF. The other 13 patients were diagnosed through CT findings. Autopsy was done for 2 of 13 patients, which shows usual interstitial pneumonia pattern accompanied with diffuse alveolar damage suggesting AE-IPF. Bronchoalveolar lavage was obtained for one patient in the tacrolimus group and one patient in the non-tacrolimus group. Endotracheal aspirate was obtained for the other 13 patients. Patients in both groups had hypoxia; The P/F ratios before the treatments were 175±77 in the tacrolimus group and 169±99 in the non-tacrolimus group, showing no significant difference between the two groups. LDH levels before treatment were elevated in both groups and showed no significance between the two groups (419±166 IU/L in the tacrolimus group and 682±353 IU/L in the non-tacrolimus group). LDH isozyme was measured 2 out of 5 patients in tacrolimus group as followings; LDH1 21.0±11.3%, LDH2 39.0±1.4%, LDH3 24.5±7.8%, LDH4 8.5±3.5%, LDH5 7.0±1.4% (High LDH2 and high LDH3 is compatible with AE-IPF. Low LDH5 is not compatible with liver origin.) Clinical and laboratory parameters measured before the initiation of treatment, including age, sex, duration since diagnosis of idiopathic pulmonary fibrosis, smoking status, score of new IPF severity classification by Japanese Ministry of Health, Labour and Welfare (classified into I, II, III, and IV mainly with cutoff value of 60, 70, 80 mmHg of oxygen pressure in artery at rest), C-reactive protein levels, and KL–6 levels, surfactant protein D (SP-D), surfactant protein A (SP-A), pH on blood gas analysis, CT score (24), and CT pattern (15) were not significantly different between the two groups (Table 1). Until acute exacerbation, no patient out of 5 tacrolimus group patients and 4 patients (one patient had prednisolone alone, and three patients had prednisolone, CyA) out of 10 non-tacrolimus group patients had had immunosuppressant (Fisher’s exact test: not significant). No patient had received pirfenidone.

Survival rate and duration

Treatment began 10.4±6.3 days after the onset of acute symptoms in the tacrolimus group and 8.3±7.7 days after the onset of acute symptoms in the non-tacrolimus group, showing no significant difference between the groups. Four patients in each group underwent mechanical ventilation during the course of their hospitalization. Four of the 5 patients in the tacrolimus group and 1 of the 10 patients in the non-tacrolimus group survived their acute episode and were discharged (p<0.05, the Fisher’s exact test; Table 2). None of the five survivors required an oxygen supply or mechanical ventilation after discharge, and none experienced re-exacerbation. The median duration of survival for the non-tacrolimus group was 38 days, whereas the median duration for the tacrolimus group was more than 92 days (p<0.05) (Fig. 1). None out of 5 tacrolimus group patients and 4 out of 10 non-tacrolimus patients had 2nd AE-IPF and eventually died.

Clinical parameters

As shown in Fig. 2-A, elevated LDH levels in the tacrolimus group were normalized (263±40 IU/L) 14 days after initiation of the treatment while LDH in the non-tacrolimus group did not change and continued to be a high level throughout the observation period. LDH levels 14 days after the initiation of the therapies showed a statistically sig-
Table 2. Clinical Course

<table>
<thead>
<tr>
<th>Group</th>
<th>Case</th>
<th>Age (year)</th>
<th>Sex</th>
<th>Ventilator</th>
<th>Outcome/ follow up duration for survivors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tacrolimus</td>
<td>1</td>
<td>75</td>
<td>F</td>
<td>2 days</td>
<td>Discharged on day 83/ 6 months.</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>72</td>
<td>F</td>
<td>No</td>
<td>Discharged on day 64/ 4 months.</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>68</td>
<td>F</td>
<td>5 days</td>
<td>Discharged on day 49/ 3 months.</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>72</td>
<td>M</td>
<td>7 days</td>
<td>Discharged on day 64/ 3 months.</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>84</td>
<td>M</td>
<td>7 days</td>
<td>Died of 1st AE on day 11</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>86</td>
<td>M</td>
<td>No</td>
<td>Died of 1st AE on day 3</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>65</td>
<td>F</td>
<td>108 days</td>
<td>Died of pneumothorax after 2nd AE day 131</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>47</td>
<td>F</td>
<td>1 day</td>
<td>Died of pneumothorax after 1st AE on day 83</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>82</td>
<td>F</td>
<td>34 days</td>
<td>Died of 2nd AE on day 55</td>
</tr>
<tr>
<td>Non-tacrolimus</td>
<td>10</td>
<td>90</td>
<td>F</td>
<td>No</td>
<td>Died of 1st AE on day 2</td>
</tr>
<tr>
<td>Tacrolimus</td>
<td>11</td>
<td>69</td>
<td>M</td>
<td>20 days</td>
<td>Died of 2nd AE on day 36</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>87</td>
<td>F</td>
<td>No</td>
<td>Died of DIC/infection during 1st AE on day 18</td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>74</td>
<td>M</td>
<td>No</td>
<td>Died of DIC/infection during 1st AE on day 27</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>73</td>
<td>M</td>
<td>No</td>
<td>Died of 2nd AE on day 42</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>78</td>
<td>F</td>
<td>No</td>
<td>Discharged on day 121/ &gt; 36 months.</td>
</tr>
</tbody>
</table>

F = female; M = male; AE = Acute Exacerbation; DIC/infection = disseminated intravascular coagulation due to infection. * All four survivors in the tacrolimus group are still alive without re-exacerbation or admission for any reason, and we have remained in periodic contact with them. † Patient 15 was followed for 36 months after discharge without re-exacerbation or admission for any reason. However, we are no longer in contact with her.

Figure 1. Kaplan-Meier curve for overall survival in both groups. Day 0 is the first day of mPSL pulse therapy. All non-survivors died of AE-IPF. P values were examined by a log-rank test. The median survival time for the tacrolimus group could not be obtained, because 4 out of 5 patients were still alive at the time of the survey. Solid line, tacrolimus group; Dotted line, non-tacrolimus group.

A significant difference between the groups. The P/F ratio in the tacrolimus group increased and normalized on day 14 and was significantly higher than that of the non-tacrolimus group on day 28 (Fig. 2-B). KL-6 levels in both groups are shown on Fig. 3. CT images after treatment was available for four patients in tacrolimus group and two patients in non-tacrolimus group, while the other nine patients died before CT reevaluation. CT score is summarized in Fig. 4. The scores of five patients were improved and these five patients survived. The other one patient with a worsened score did not survive. All four patients with CT reevaluation in tacrolimus group had a better score in CT reevaluation and these four patients survived.

No patient in the tacrolimus group died of side effects, and four out of 10 patients in non-tacrolimus groups died of side effects (two of pneumothorax and two of infection) (Fisher’s exact test: not significant). In the tacrolimus group, major side effects including fungal pneumonia, mediastinal emphysema (case 1), Candida dermatitis (case 2), and steroid psychosis (case 3), were observed; all patients with such side effects recovered with appropriate treatment. In the non-tacrolimus group, major side effects including pneumothorax (cases 7, 8), disseminated intravascular coagulation due to infection (cases 12, 13), steroid psychosis (cases 11, 14), and compression fracture of lumbar spine (case 15) were observed. Patients 7, 8, 12, and 13 died of side effects.

Discussion

Here we have shown a higher survival ratio and longer survival duration in AE-IPF patients treated with a combination therapy of tacrolimus and mPSL pulse therapy (Fig. 1). Major clinical parameters such as LDH, and P/F ratio improved dramatically in the tacrolimus group. We concluded that the observed differences primarily resulted from the addition of tacrolimus to the conventional therapeutic regimen, because the diagnosis of AE-IPF was clear for patients in both groups and there were no significant baseline differences between the two groups.

Diffuse alveolar damage, a pathological finding in AE-IPF, has been subdivided into three sequential phases (2, 25): an exudative phase marked with hyaline membrane, a proliferative phase distinguished by fibrogenesis, and a fibrotic phase characterized by remodeling. During the proliferative and fibrotic phase, fibrocytes can be recruited in response to chemokines and may potentiate fibrogenesis via extracellular matrix production and/or secretion of a profibrotic factor (26, 27).

Corticosteroid therapy has limited effectiveness in acute respiratory distress syndrome (28-30). It accelerates the resolution of both systemic inflammation and peripheral acquired corticosteroid resistance (31). Acute respiratory dis-
tress syndrome, whose pathological pattern is diffuse alveolar damage likewise, includes most of AE-IPF by definition, which is the reason why corticosteroid therapy is often selected to treat AE-IPF despite the lack of straightforward evidence of its effectiveness in AE-IPF. Despite effectiveness of corticosteroid single therapy for ARDS, patients in tacrolimus group had significantly better improvement of LDH value and P/F ratio on day 14 in our study, which means that tacrolimus treatment is more effective for acute phase of AE-IPF. Though corticosteroid therapy was effective to some extent for acute phase diffuse alveolar damage, four patients of non-tacrolimus group in our study suffered from re-exacerbation, which is compatible with previous study (7, 17) and there is no evidence showing that corticosteroid alone could prevent the fibrotic phase featured by remodeling. The anti-inflammatory effect of tacrolimus, by inhibiting calcineurin, might prevent two unfavorable consequences: re-exacerbation and fibrotic change. In particular, the pathway of tacrolimus inhibiting transforming growth factor beta might suppress fibrotic process (32). As a result, our four patients in the tacrolimus group, who survived the first 14 days (exudative and proliferative phase) could avoid both the re-exacerbation and fibrotic phase thereafter.

Tacrolimus protocols now well established for primary immunosuppressive therapy in kidney and liver transplantation in place of CyA. Compared with CyA, post-transplantation immunosuppressive management with tacrolimus improved acute rejection ratio after renal trans-

Figure 2. (A) Serum LDH levels in both groups. Day 0 is the first day of mPSL pulse therapy. Error bars show the standard deviation from the mean. Horizontal bars show the normal range of LDH (114-265 IU/L). The number of patients in both groups decreased over time due to patient death. P values were examined by the Mann-Whitney test (* < 0.05, ** < 0.01). (B) P/F ratio in both groups. Day 0 is the first day of mPSL pulse therapy. Error bars show standard deviation from the mean. The number of patients in both groups decreased over time due to patient death. P values were examined by the Mann-Whitney test. Solid line, tacrolimus group; Dotted line, non-tacrolimus group.

Figure 3. KL-6 in both groups. Day 0 is the first day of mPSL pulse therapy. Each group contains four patients, because the other patients died before KL-6 re-evaluation. Error bar shows the standard deviation from the mean. Horizontal bar shows the upper limit of normal range (500 U/mL). P values were examined by the Mann-Whitney test. Solid line, tacrolimus group; Dotted line, non-tacrolimus group.

Figure 4. CT scores of six patients before and after treatment. This score ranges from 0 to 100, and higher score means severe IPF. ◆: four survivors of tacrolimus group, □: one survivor of non-tacrolimus group, ■: one non-survivor of non-tacrolimus group.
plantation (33–35), mortality, retransplantation, and treatment failure for immunological reason after liver transplantation (36). Experience with immunosuppressive management with tacrolimus for lung transplantation is rapidly accumulating, which shows less acute rejection and less bronchiolitis obliterans syndrome than CyA protocol (37, 38). Tacrolimus is not only more effective than CyA, but it is also as safe as CyA (22, 36, 37). These data on organ transplantation may support the possible advantage of tacrolimus over CyA to treat AE-IPF. Although tacrolimus is more dia-
etogenic (36, 37), diabetes is a controllable side effect.

The present study had several limitations. First, it was a retrospective study of a small number of patients. While AE-IPF is clinically important, its rarity makes it difficult to study in a large number of patients. Observed survival ratio and survival duration were not well confirmed due to the lack of cases. But the chance for α-error (false positive) of significant survival difference among both groups is less than 5% in this study, because we have defined α value as 0.05 and p value was less than α.

The authors state that they have no Conflict of Interest (COI).

Acknowledgement

No grant was offered for this study.

References

1. American Thoracic Society/European Respiratory Society Interna-
15. Akira M, Kozuka T, Yamamoto S, Sakatani M. Computed tomo-