Bolus infusion of human urinary trypsin inhibitor improves intractable interstitial pneumonia in patients with connective tissue diseases

S. Tsujimura, K. Saito, S. Nakayamada and Y. Tanaka

Objective. Interstitial pneumonia (IP) associated with CTDs often progresses despite conventional immunosuppressive treatment. We investigated the efficacy of human urinary trypsin (UT) inhibitor (ulinstatin) on refractory IP.

Methods. Five patients with IP received UT inhibitor (3 × 10^5 U) infusion into the internal jugular vein, three times in a single day. The response to this therapy was assessed clinically and by chest CT, PaO_2 and serum KL-6. The kinetics of UT inhibitor was determined in arterial blood. We measured serum levels of monocyte chemotactic protein-1 and TGF-β1, which are thought to be involved in the pathogenesis of IP.

Results. Serum concentrations of UT inhibitor increased immediately to >150 U/ml after infusion of 3 × 10^5 U of UT inhibitor. The treatment resulted in clinical and radiological improvements in four patients, and allowed reduction of oxygen therapy following improvement of hypoxaemia within 1 month. UT inhibitor decreased serum levels of KL-6 in all patients and had no adverse effects. MCP-1 and TGF-β1 concentrations were higher in the patients than in normal subjects, and infusion of 3 × 10^5 U of UT reduced the concentrations within 3 h of infusion.

Conclusion. UT inhibitor bolus infusion therapy is a potentially useful therapeutic strategy for intractable IP based on the different mechanism of action relative to conventional immunosuppressive therapy and lack of serious treatment-related adverse effects.

Key words: Human urinary trypsin inhibitor, Interstitial pneumonia, Connective tissue diseases.

Introduction

Interstitial pneumonia (IP) is a serious complication in patients with systemic autoimmune diseases, and sometimes progresses rapidly causing death in some patients [1, 2]. Although immunosuppressive-cytotoxic agents, such as AZA, CSA, tacrolimus and cyclophosphamide, are used in combination with corticosteroids in patients with refractory IP [3–5], their efficacy for IP is controversial [2, 6]. In addition, these agents are not always tolerated because of various adverse effects including opportunistic infections [7–9]. In such cases, intensive immunosuppressive therapy cannot be continued, and measures to treat patients with repeated opportunistic infection are needed.

The pathological process of IP is accelerated by various inflammatory factors at the loci of interstitial inflammation. Ulinastatin, a human urinary trypsin (UT) inhibitor, is a natural inhibitor of protease and frequently used for the treatment of shock [10] and acute pancreatitis [11]. Since UT inhibitor is also known to inhibit various inflammatory factors associated with the development and progression of IP, such as cytokines [12], oxygen radicals [13] and adhesion molecules [14], it is possible that UT inhibitor could be therapeutically useful against active IP. In fact, we have reported that bolus infusions of UT inhibitor improved severe IP in MCTD [15]. However, the efficacy of UT inhibitor in IP with CTDs has not been established. In present study, we used UT inhibitor in bolus infusion to treat five patients with active IP who did not respond to immunosuppressive therapy or developed adverse effects, and evaluated the efficacy and safety of such treatment.

Patients and methods

Patients

Five patients with IP complicating systemic autoimmune diseases [one with MCTD, two with SSc, one with microscopic polyangiitis (MPA), and one with DM], who were admitted to our university hospital between July 2002 and June 2004, were included in this study (Table 1). The diagnosis of MCTD was based on the criteria described by Alarcon-Segovia and Villarreal [16]. SSc was diagnosed according to the ACR criteria [17]. The Chapel Hill nomenclature was used to define MPA [18]. DM was diagnosed according to the criteria of Bohan and Peter [19]. IP in the five patients fulfilled all the following criteria: (i) IP was associated with CTDs, not with drugs or infection; (ii) IP was progressively assessed by symptoms including dyspnoea, progressive decrease in partial pressure of oxygen in arterial blood (PaO_2) and worsening of chest radiographic findings; and (iii) complications apart from IP were controllable but IP was not suppressed sufficiently by immunosuppressants and/or could not be treated further with immunosuppressants due to adverse effects. IP in four patients progressed in spite of intensive immunosuppressive treatment and resulted in severe Candida pneumonia (Patient 1), acute pancreatitis (Patient 2), Pneumocystis pneumonia (PCP) (Patient 3) and pandemic cingulum (Patient 5). IP in Patient 4 could not be resolved with CSA plus intravenous cyclophosphamide pulse therapy (IVCY), and finally resulted in acute exacerbation of IP. The use of UT inhibitor bolus infusion therapy was approved by the ethics committee of our institution and informed consent was obtained from the patients.

Assessment of IP

The severity of IP was assessed by chest CT, PaO_2 and serum levels of KL-6. KL-6 is a mucinous glycoprotein expressed on type II pneumocytes and used clinically as a marker of IP in various collagen diseases and of pulmonary interstitial damage [20, 21]. Serum KL-6 levels increase with deterioration of IP, while successful treatment of IP results in significant falls in these levels [21]. Serum KL-6 concentrations were measured by electrochemiluminescence immunoassay (Eisai, Tokyo, Japan).
UT inhibitor bolus infusion therapy

UT inhibitor (ulinastatin, Miracrid® Motida Ltd, Japan) was infused into the internal jugular vein, rather than a peripheral vein, using a central venous catheter in order to obtain a high UT inhibitor concentration in the lung. A single course of UT inhibitor bolus infusion therapy consisted of bolus infusion of $3 \times 10^5$ U of UT inhibitor repeated three times at 5-h intervals (total dose: $9 \times 10^5$ U/day). After the bolus infusion, the dose of prednisolone (PSL) was not changed but no new immunosuppressants were added for 1 month.

Kinetics of UT inhibitor concentration in arterial blood

We measured the serum concentrations of UT inhibitor by radioimmunoassay (SBS, Kanagawa, Japan) in arterial blood samples obtained from the brachial artery of three patients to evaluate UT inhibitor concentration in the pulmonary circulation over a period of 60 min after initial bolus infusion of $3 \times 10^5$ U of UT inhibitor.

Measurements of monocyte chemotactic protein-1 and TGF-β1

We collected arterial blood samples from the brachial artery of six normal volunteers and patients to measure the levels of monocyte chemotactic protein (MCP-1) and TGF-β1 in the pulmonary circulation. Informed consent was obtained from all the donors who were enrolled in the study. The serum was separated rapidly from the collected arterial blood (within 15 min after collection), and MCP-1 and TGF-β1 were measured with an enzyme immunoassay system (SBS).

Statistical analysis

Values are expressed as mean±s.d. Student’s t-test was used to compare data between the two groups. A P-value <0.05 denoted the presence of statistically significant difference.

Results

Kinetics of UT inhibitor concentration in arterial blood

Arterial blood samples were collected from the brachial artery over a period of 60 min and the serum concentrations of UT inhibitor were measured. UT inhibitor concentrations were $>150$ U/ml at 20 min and remained at more than $100$ U/ml at the end of 60 min after a single bolus infusion of $3 \times 10^5$ U of UT inhibitor (Fig. 1).

Clinical efficacy of UT inhibitor bolus infusion therapy

Patient 1 was diagnosed as MCTD with IP, and intensive immunosuppressive treatment with high-dose corticosteroids and CSA or repeated IVCY did not resolve the progressive IP for 7 yrs, and finally resulted in severe Candida pneumonia. Infusion of amphotericin B improved Candida pneumonia, but hypoxaemia worsened with progression of CT-documented MCTD-related IP. However, additional immunosuppressive therapy was considered inappropriate in the presence of the fungal infection. Accordingly, we used UT inhibitor bolus infusion therapy. This resulted in the attenuation of the Velcro rales and improvement of ground-glass attenuation on the chest X-ray. Two weeks after a single course of UT inhibitor bolus infusion therapy, the patient showed relief from dyspnoea and a decrease in all-day persistent cough to a few episodes per day. Moreover, serum level of KL-6 fell from $8253$ to $5841$ U/ml and PaO₂ increased to 80 mmHg. Six months after nine courses of UT inhibitor bolus infusion therapy once every month, she was maintained on only 7 mg/day of PSL when KL-6 decreased to 938 U/ml (Fig. 2A) [15].

Patient 2 was admitted to our hospital for exacerbation of IP after 8-yr corticosteroid therapy for idiopathic IP, combined with 2-yr home oxygen therapy. After admission, she was diagnosed as SSc with IP, based on the presence of RP, severe skin sclerosis and the gastrointestinal tract manifestations. Despite the addition of CSA, the ground-glass attenuation increased on chest X-ray and CT, and then she developed acute pancreatitis. Pancreatitis responded well to infusion of antibiotics and withdrawal of CSA. UT inhibitor bolus infusion therapy was applied for progression of IP. The treatment resulted in attenuation of the...
ground-glass appearance, improvement of PaO2, allowing a reduction in oxygen inhalation to 1.01/min. After two courses of UT inhibitor bolus infusion therapy, KL-6 decreased to 403 U/mL. She was maintained on only 5 mg/day of PSL at discharge from the hospital (Fig. 2B).

Patient 3 was diagnosed as MPA, based on IP, rapid progressive glomerulonephritis and high titre of myeloperoxidase-specific ANCA. Despite treatment with oral cyclophosphamide, high-dose corticosteroids and methylprednisolone pulse infusion, IP recurred several times during a period of 8 yrs and finally resulted in PCP. Although PCP responded to pentamidine, MPAbolus infusion therapy. The PaO2 and serum levels of KL-6 improved significantly relative to the corresponding values before infusion (Fig. 3).

Figure 4 shows CT findings of the five patients before and after treatment. The findings improved markedly in Patients 1, 3 and 5 after UT inhibitor bolus infusion therapy. Furthermore, the density of ground grass attenuation decreased gradually in Patient 4, while pulmonary fibrosis in Patient 2 was almost unchanged at 1 month after a single course of UT inhibitor bolus infusion therapy.

Effects of UT inhibitor bolus infusion therapy on MCP-1 and TGF-β1

Since UT inhibitor is known to have anti-inflammatory and organ-protective effects through the inhibition of various inflammation-related mediators, we examined whether UT inhibitor bolus infusion therapy influenced the production of MCP-1 and TGF-β1, which are both reported to be associated with the progression of IP [22, 23]. Before treatment, serum concentrations of MCP-1 and TGF-β1 were significantly higher in patients with IP than the normal control (Fig. 5). However, the initial bolus infusion of 3 × 10^6 U UT inhibitor resulted in a significant decrease in serum MCP-1 level measured at 3 h after infusion and also tended to decrease serum TGF-β1 concentration albeit insignificantly.

Discussion

Progressive IP is a life-threatening manifestation of autoimmune disease and often intractable despite concomitant therapy with high-dose corticosteroids and immunosuppressants [1, 2]. In addition, repeated intensive immunosuppressive therapy including high-dose corticosteroids often causes treatment-related severe adverse effects such as opportunistic infections like fungal, pneumocystis and CMV pneumonia [7–9]. Once patients develop such complication of severe infections, the use of additional immunosuppressants is inappropriate even when IP is not controlled well. We presented three patients who showed resistance to various combination therapies of corticosteroid and immunosuppressants including repeated IVCY, and finally developed severe opportunistic pneumonias. However, UT inhibitor bolus infusion therapy successfully improved refractory IP, without serious adverse effects such as bone marrow suppression,
organ failure and opportunistic infection. Furthermore, repeated UT inhibitor bolus infusion therapy maintained the state of IP remission and allowed tapering of steroids in Patients 1 [15], 2, 3 and 5.

The pathological changes associated with IP include inflammatory cell accumulation and alveolar damage together with progressive fibrosis and remodelling of the bronchoalveolar tree. These processes are reported to involve various mediators, such as

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**Fig. 2.** Clinical course of the five patients. (A) Case 1 [19]. (B) Case 2. (C) Case 3. (D) Case 4. (E) Case 5. Open arrows: single IVCY, closed arrows: one course of UT inhibitor bolus infusion therapy. CPA: oral cyclophosphamide; O₂: oxygen inhalation; Candida: fungal pneumonia with Candida albicans; CMV: cytomegalovirus viremia; PaO₂: partial pressure of oxygen in arterial blood of Cases 1, 3, 4 and 5 on breathing room air and Case 2 during breathing of 1.0 l/min of oxygen.
oxygen radicals [24], MCP-1 [22], TGF-β1 [23], matrix metalloproteinase (MMP) [25], and adhesion molecules [26]. UT inhibitor is a proteoglycan that increases in response to various injuries in the human body [27, 28]. UT inhibitor is often used for life-threatening inflammation such as acute pancreatitis and acute circulatory collapse. It has anti-inflammatory and organ-protective effects through inhibition of various inflammation-related mediators including proteases [29], cytokines [12], oxygen radicals [13] and adhesion molecules [14]. It has been reported that MCP-1 and TGF-β1 produced by type II alveolar epithelial cells are increased in bronchoalveolar lavage fluid of patients with IP [30, 31]. MCP-1 induces extravascular infiltration of neutrophils, macrophages and TH2 type of lymphocytes. TGF-β1 is also involved in the pathogenesis of IP by up-regulating the proliferation of pulmonary fibroblasts. Actually, in our patients, we observed high levels of MCP-1 and TGF-β1 and the values decreased at 3 h after the initial bolus infusion of UT inhibitor. Others have reported that UT inhibitor provides protection of alveolar epithelial cells by suppressing the release of oxygen radicals from neutrophils, inhibits accumulation of inflammatory cells by suppressing the production of TNF-α and IL-8 and down-regulating the expression of intercellular adhesion molecule-1 (ICAM-1) on endothelial cells, and could be effective in preventing pulmonary remodelling by suppressing the production of MMP. Thus, these mechanisms of UT inhibitor seem different from those of conventional immunosuppressive, cytotoxic agents. Furthermore, the present cases emphasized the usefulness of UT inhibitor bolus infusion therapy for IP that could not be controlled by conventional therapies.

Although others have suggested that UT inhibitor is effective for paraquat-induced pulmonary fibrosis and idiopathic IP [32, 33], there is no convincing report of the therapeutic efficacy of UT inhibitor for IP with systemic autoimmune diseases. Kamei et al. [33] reported that intravenous infusion of UT inhibitor (1.5 \times 10^5 \text{ U/mL} \times 2/day for 5 days) had no significant effects on ICAM-1, IL-8 and difference of alveolar arterial oxygen partial pressure (AaDO2). One of the reasons for the inadequate effect of UT inhibitor infusion is that the UT inhibitor blood concentration scarcely reaches an effective level because the half-life of UT inhibitor is only 40 min [34]. Others have reported that suppression of production of lipopolysaccharide (LPS)-induced oxygen radicals and cytokines requires more than 30 and 100 U/ml UT inhibitor concentrations, respectively [35, 36]. In addition, concentrations well above 40 U/ml of UT inhibitor are required to suppress MMP production [29]. However, it is almost impossible to obtain these concentrations of UT inhibitor by conventional intravenous infusion through peripheral veins even in the case of 3 \times 10^5 \text{ U} of UT inhibitor bolus infusion. Sugiura et al. [37] reported that UT inhibitor concentration was 91.5 U/ml at only 2 min and fell to 52.6 U/ml at 30 min after intravenous bolus infusion of 3 \times 10^5 \text{ U} of UT inhibitor through peripheral veins. It is notable that we overcame this issue by bolus infusion of UT inhibitor into the superior vena cava using a central venous catheter. The selection of this route ensured sufficient concentrations of UT inhibitor in the pulmonary

### Table 2. Efficacy of a course of UT inhibitor bolus infusion on IP

<table>
<thead>
<tr>
<th>Case 1</th>
<th>Case 2</th>
<th>Case 3</th>
<th>Case 4</th>
<th>Case 5</th>
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<tbody>
<tr>
<td>Combination therapy</td>
<td>PSL 60 mg</td>
<td>PSL 10 mg</td>
<td>PSL 2.5 mg</td>
<td>–</td>
</tr>
<tr>
<td>Dyspnoea</td>
<td>Improved</td>
<td>Decreased</td>
<td>No change</td>
<td>Improved</td>
</tr>
<tr>
<td>Velcro rales</td>
<td>Decreased</td>
<td>Decreased</td>
<td>No change</td>
<td>Decreased</td>
</tr>
<tr>
<td>Oxygen supply (l/min)</td>
<td>Before 2.0, 1.5, 1.0, 1.5, 1.0</td>
<td>1 Month after 0, 1.0, 0, 1.0, 0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LDH (U/mL)</td>
<td>208, 165, 189, 212, 250</td>
<td>83, 120, 84, 69, 92</td>
<td></td>
<td></td>
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<tr>
<td>PaO₂ (mmHg)</td>
<td>5392, 543, 666, 2982, 1274</td>
<td></td>
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<tr>
<td>KL-6 (U/mL)</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Adverse effects</td>
<td>–</td>
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**P < 0.01, by paired t-test.**
circulation (Fig. 1), and probably resulted in reduction of various mediators at the site of pneumonitis.

In conclusion, UT inhibitor bolus infusion therapy may be useful for IP based on its anti-inflammatory and anti-oxidant effects, and on lacking serious adverse effects, such as bone marrow suppression, organ failure and opportunistic infection. However, more cases need to be reported before the establishment of UT inhibitor bolus infusion therapy. We propose that UT inhibitor bolus infusion therapy is potentially useful as an alternative therapy for refractory IP in patients resistant or intolerable to conventional immunosuppressive treatments.

<table>
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<tr>
<th>Rheumatology key messages</th>
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<tr>
<td>UT inhibitor bolus infusion therapy could show anti-inflammatory and anti-oxidant effects without serious adverse effects.</td>
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<tr>
<td>UT inhibitor bolus infusion therapy could be useful as an alternative therapy for refractory IP.</td>
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References


