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CASE REPORT

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Human urinary trypsin inhibitor bolus infusion improved severe interstitial pneumonia in mixed connective tissue disease

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Abstract Interstitial pneumonia (IP) with mixed connective tissue disease (MCTD) often progresses despite immunosuppressive therapies that caused serious infections. Human urinary trypsin inhibitor (UT) inhibits inflammatory factors associated with IP, without immunosuppression. UT bolus infusion rescued a female MCTD patient with refractory IP and severe opportunistic fungal pneumonia. Her IP diminished with monthly UT bolus infusion despite tapering of prednisolone, without UT-related side effects. UT pulse therapy could prove beneficial for refractory IP in MTCD even with opportunistic infections.

Key words Human urinary trypsin inhibitor (UT) · Mixed connective tissue disease (MCTD) · Opportunistic infection · Refractory interstitial pneumonia (IP)

Introduction

Interstitial pneumonia (IP) represents a serious complication in patients with systemic autoimmune diseases, and sometimes progresses rapidly causing death in some patients.[1,2] Although immunosuppressive-cytotoxic agents, which are known to target inflammatory cells, such as azathioprine, cyclosporine (CsA), tacrolimus, and cyclophosphamide, are used together with corticosteroids in patients with corticosteroid-resistant IP,[3–5] their efficacy for IP is controversial.[6,7] In addition, these agents are not always tolerated because of various adverse effects including opportunistic infections.[8–11] We often experience IP that is not suppressed sufficiently by immunosuppressive treatments and cannot keep administering immunosuppressive treatments because of adverse effects. Therefore, the development of the treatment for such refractory IP is important.

The pathological processes of IP are accelerated by various inflammatory factors at the loci of interstitial inflammation. Ulinastatin, a human urinary trypsin inhibitor (UT), is a natural inhibitor of protease that is frequently used for the treatment of shock[12] and acute pancreatitis.[13] Because UT has also been known to inhibit various inflammatory factors associated with the development and progression of IP, such as tumor necrosis factor (TNF)-α,[14,15] oxygen radicals,[16] intercellular adhesion molecule-1 (ICAM-1),[17,18] and matrix metalloproteinase (MMP),[19] UT could be useful for the treatment of active IP. To our knowledge, however, there are no reports regarding the efficacy of UT in IP with rheumatic diseases. We report here a case of refractory IP associated with mixed connective tissue disease (MCTD) in a patient who was successfully treated without increased risk of infection by UT bolus infusion.

Case report

A 33-year-old woman was diagnosed with MCTD in 1995, based on Raynaud's phenomenon, an extremely high titer (>200 index) of antinuclear ribonucleoprotein (nRNP) antibody, polyarthritis, erythema, myopathy with elevated creatine kinase (CK), marked dilatation of the esophagus, and IP. Computed tomography (CT) revealed severe IP with diffuse ground-glass attenuation and patchy areas of consolidation. Histopathological examination of lung biopsy obtained by video-assisted thoracic surgery showed usual interstitial pneumonia (UIP) and bronchiolitis obliterans-organizing pneumonia (BOOP). The initial treatment of 0.8 mg/kg body weight (BW) per day of oral prednisolone (PSL) combined with 150 mg/day of CsA was effective for almost all the symptoms and signs. However, this combination treatment was not sufficient to suppress IP. Moreover, CsA had to be withdrawn because of renal dysfunction. Therefore, ten courses of intravenous cyclophosphamide pulse therapy (IVCY; 1 g/day) were performed, and PSL

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was tapered to 0.4 mg/kg BW per day, but her IP soon worsened again. Three courses of methyl-PSL pulse therapy (1 g/day for 3 days) and PSL 1.0 mg/kg BW per day with 75 mg/day of CsA was further required for the improvement of IP. Interstitial pneumonia was prevented from getting worse, and PSL was tapered carefully. However, the patient continued to have persistent dry cough and dyspnea over this period, and serum levels of KL-6 remained at around 4000 U/ml (normal range 105–401 U/ml). In June 2002, while on 0.2 mg/kg BW per day of oral PSL and 75 mg/day of CsA, the dry cough and dyspnea began to get worse once more, and ground-glass attenuation expanded on chest X-ray. Although PSL was immediately increased to 0.5 mg/kg BW per day, the patient was not able to talk while walking due to dyspnea and the dry cough continued for hours; moreover, the titer of KL-6 became elevated suddenly (Fig. 1A). In July, she was admitted to the casualty department of our hospital for acute exacerbation of IP.

On admission, the body temperature was 35.6°C, pulse 86 beats/min, blood pressure 124/82 mmHg, and respiratory rate 38/min. Facial features were typical of pink puffers. Chest auscultation revealed marked Velcro rales in the mid-to-lower lung fields bilaterally. Laboratory tests showed hypoxemia, a restrictive ventilatory pattern, and abnormal alveolar capillary gas diffusion with extremely high serum levels of KL-6 and surfactant protein D (Table 1). No bacteria, fungus, or virus was detected in the sputum samples obtained on several occasions. Chest X-ray and CT revealed severe IP (Figs. 2A and 3A) and gallium-citrate scintigraphy showed markedly abnormal uptake in the middle and lower lungs bilaterally (Fig. 4A).

Based on these findings, the patient was treated with IVCY (10 mg/kg i.v.) and oral CsA was withdrawn because it had been ineffective at that time. Two days after IVCY, she developed severe *Candida* pneumonia with high fever, wheezing, and moist rales. *Candida albicans* was detected in the sputum and β-D-glucan became positive. Intravenous infusion of 0.5 mg/kg per day of amphotericin B resulted in improvement of *Candida* pneumonia, but hypoxemia worsened with progression of MCTD-related IP documented by CT, and the serum titer of KL-6 remained at an extremely high level (9305 U/ml; Fig. 1A). Additional aggressive immunosuppressive treatments such as methyl-PSL pulse therapy and IVCY appeared to be critical in the development of severe fungal infection. Thus, the dosage of PSL was increased to 1.0 mg/kg per day, although 2 weeks later this dose proved to be insufficient to suppress IP on chest X-ray and CT (Figs. 2B and 3B). The dyspnea and Velcro rales did not improve and PaO_2 ranged from 50 to 60 mmHg on room air, while the titer of KL-6 was still 8356 U/ml.

A decision was made to treat the patient with UT based on the hypothesis that UT would inhibit IP-related inflammatory factors including TNF-α,[14,15] oxygen radicals,[16] ICAM-1,[17,18] and MMP,[19] without clinical immunosuppression. Human urinary trypsin inhibitor infusion was approved by the Human Research Committee of our institution, and informed consent was obtained from the patient. Accordingly, 3×10^5 units of UT were infused into the internal cervical vein via a central venous catheter with the aim of obtaining a high UT concentration in the lung. We measured the serum UT concentrations by radioimmunoassay (SBS, Kanagawa, Japan) in several arterial blood samples obtained from the brachial artery to evaluate UT concentration in the pulmonary circulation. After the 3×10^5 units of UT infusion into the internal cervical vein, UT concentration immediately increased to >100 U/ml and remained stable at >50 U/ml after 60 min (Fig. 5). Urinary trypsin inhibitor bolus infusion was applied at 3×10^5 units of UT and was administered three times per day at 5-h intervals (9×10^5 units/day).

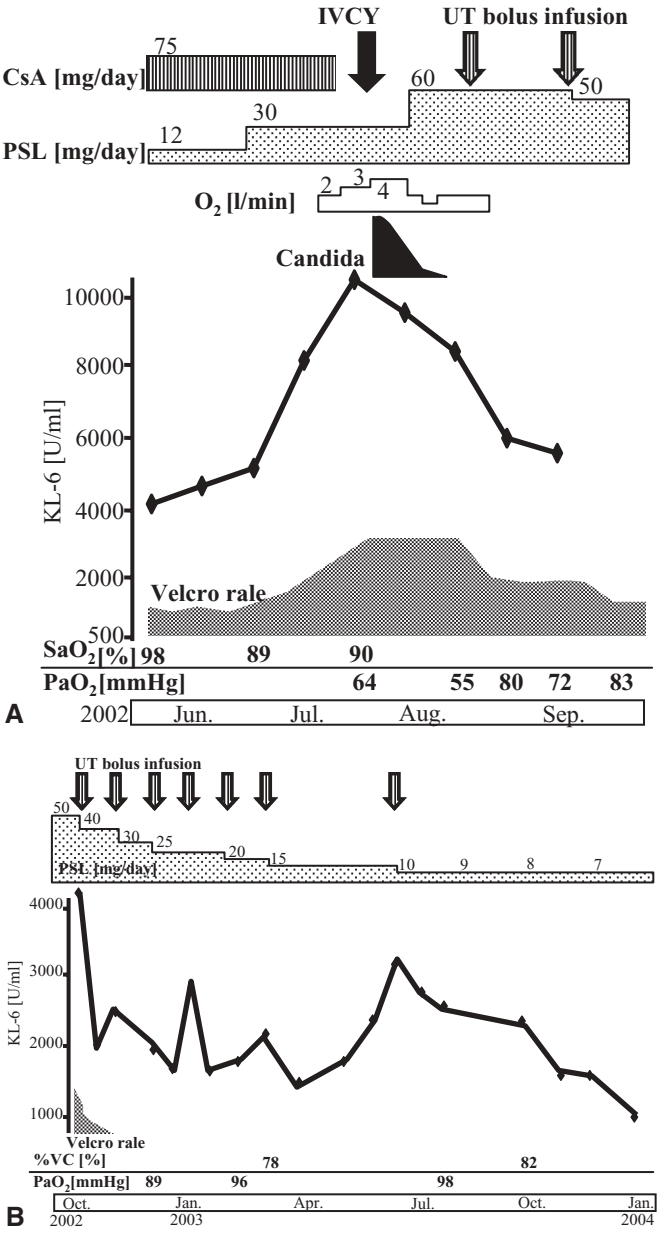

Fig. 1A,B. Clinical course. **A** Course of acute stage. **B** Long-term course. *CsA*, cyclosporin A; *IVCY*, intravenous cyclophosphamide pulse therapy; *UT*, human urinary trypsin inhibitor; *PSL*, prednisolone (or equivalent); O_2, oxygen inhalation; *Candida*, fungal pneumonia with *Candida albicans*; SaO_2, arterial blood oxygen saturation; PaO_2, arterial blood oxygen pressure; *%VC*, percent vital capacity

Table 1. Laboratory findings on admission

		Serology	
Urine			
Protein	(–)	Total protein	7.0 g/dl
Sugar	(–)	ALT	18 IU/l
Occult blood	(–)	AST	19 IU/l
Cast	(–)	LDH	307 IU/l
CBC		Creatinine	0.8 mg/dl
Leukocytes	6000/μl	CK	46 IU/l
Hemoglobin	15.0 g/dl	CRP	0.3 mg/dl
Platelets	18.7×10^4/μl	CH_{50}	25 U/ml
ESR	11 mm/h	ANA	5120 (speckled pattern)
Arterial blood gases; room air (O_2 3 l/min)			
PaO_2	64 mmHg (93 mmHg)	Anti-nRNP antibody	>200 index
$PaCO_2$	34 mmHg (37 mmHg)	KL-6	10 200 U/ml
Lung function		SP-D	1540 ng/ml
$FEV_{1\%}$	85%	Endotoxin	(–)
%VC	71%	β-D-glucan	(–)
DLco	7.59 ml/min/mmHg	Cytomegalovirus antigen	(–)
%DLco	35%		

CBC, complete blood count; ESR, erythrocyte sedimentation rate; O_2, oxygen inhalation; $FEV_{1\%}$, percent forced expiratory volume in 1 s; %VC, percent vital capacity; DLco, diffusing capacity for carbon monoxide; %DLco, percent DLco; ALT, alanine aminotransferase; AST, aspartate aminotransferase; LDH, lactic dehydrogenase; CK, creatine kinase; CRP, C-reactive protein; CH_{50}, 50% hemolytic complement activity; ANA, antinuclear antibodies; nRNP, nuclear ribonucleoprotein; SP-D, surfactant protein D

After the first UT bolus infusion, the Velcro rales and ground-glass attenuation on chest X-ray began to diminish. Two weeks after UT bolus infusion, relief from dyspnea was achieved and all-day persistent cough decreased to a few episodes per day. Moreover, the serum level of KL-6 fell to 5841 U/ml and PaO_2 increased to 80 mmHg (Fig. 1A). One month after the initiation of UT therapy, the areas of ground-glass attenuation markedly diminished on chest X-ray and CT (Figs. 2C and 3C) and the serum level of KL-6 decreased to 5392 U/ml (Fig. 1A), thus allowing the patient's discharge from the hospital. The clinical course confirmed the efficacy of UT pulse therapy, prompting us to repeat UT pulse once every month. Consequently, marked improvement of IP was noted in clinical symptoms, accompanied by improvement of pulmonary function and PaO_2 (Fig. 1B) and improvement of gallium-citrate uptake scintigraphy (Fig. 4B). Furthermore, the ground-glass attenuation areas almost disappeared on chest X-ray and CT, and serum levels of KL-6 decreased despite the tapering of PSL, without any remarkable side effects related to UT. Moreover, after nine courses of UT bolus infusion, IP never relapsed while the patient was maintained on only 7 mg/day of PSL. Six months after nine courses of UT pulse therapy, KL-6 decreased to 938 U/ml, and IP remained in remission as confirmed clinically and radiologically by chest X-ray and CT (Figs. 2D and 3D). The patient reported being able to go up and down the stairs of a stadium without dyspnea and to cheer with a loud voice.

Discussion

Interstitial pneumonia associated with systemic autoimmune diseases can be fatal even in patients treated with high-dose corticosteroids.[1,2] Combination of the latter with immunosuppressive agents such as CsA and IVCY appears to produce effective results in patients with progressive IP refractory to conventional therapies.[3–5] However, even these treatments sometimes fail to suppress rapidly progressive IP.[6,7] Moreover, repeated high-dose corticosteroid therapy combined with other immunosuppressants induce treatment-related severe adverse effects such as opportunistic infections, and could make any more aggressive immunosuppressive treatments impossible.[8–11] Indeed, our patient showed resistance to various combination therapies of corticosteroid and immunosuppressants including even repeated IVCY and finally developed severe opportunistic infection. However, the UT bolus infusion successfully rescued the patient and induced remission of IP even while the corticosteroid was tapered.

Human urinary trypsin inhibitor is a proteoglycan that increases in response to various injuries in the human body.[20,21] Urinary trypsin inhibitor is often used for life-threatening inflammation such as acute pancreatitis and acute circulatory collapse. It has anti-inflammatory and organ-protective effects by inhibition of various inflammation-related mediators including TNF-α,[14,15] oxygen radicals,[16] ICAM-1,[17,18] and MMP.[19] The pathological changes associated with IP include inflammatory cell accumulation and alveolar damage together with progressive fibrotic process and remodeling of the bronchi–alveoli. These processes are reported to involve TNF-α,[22] oxygen radical,[23,24] ICAM-1,[25] and MMP.[26] Moreover, inhibition of TNF-α prevents the progression of IP associated with rheumatoid disease.[27] Therefore, the anti-inflammatory and organ-protective properties of UT can be employed for the treatment of IP. Although some reports have suggested that UT appears to be effective for IP,[28,29] there is no convincing report of the therapeutic efficacy of UT for IP. Kamei et al.

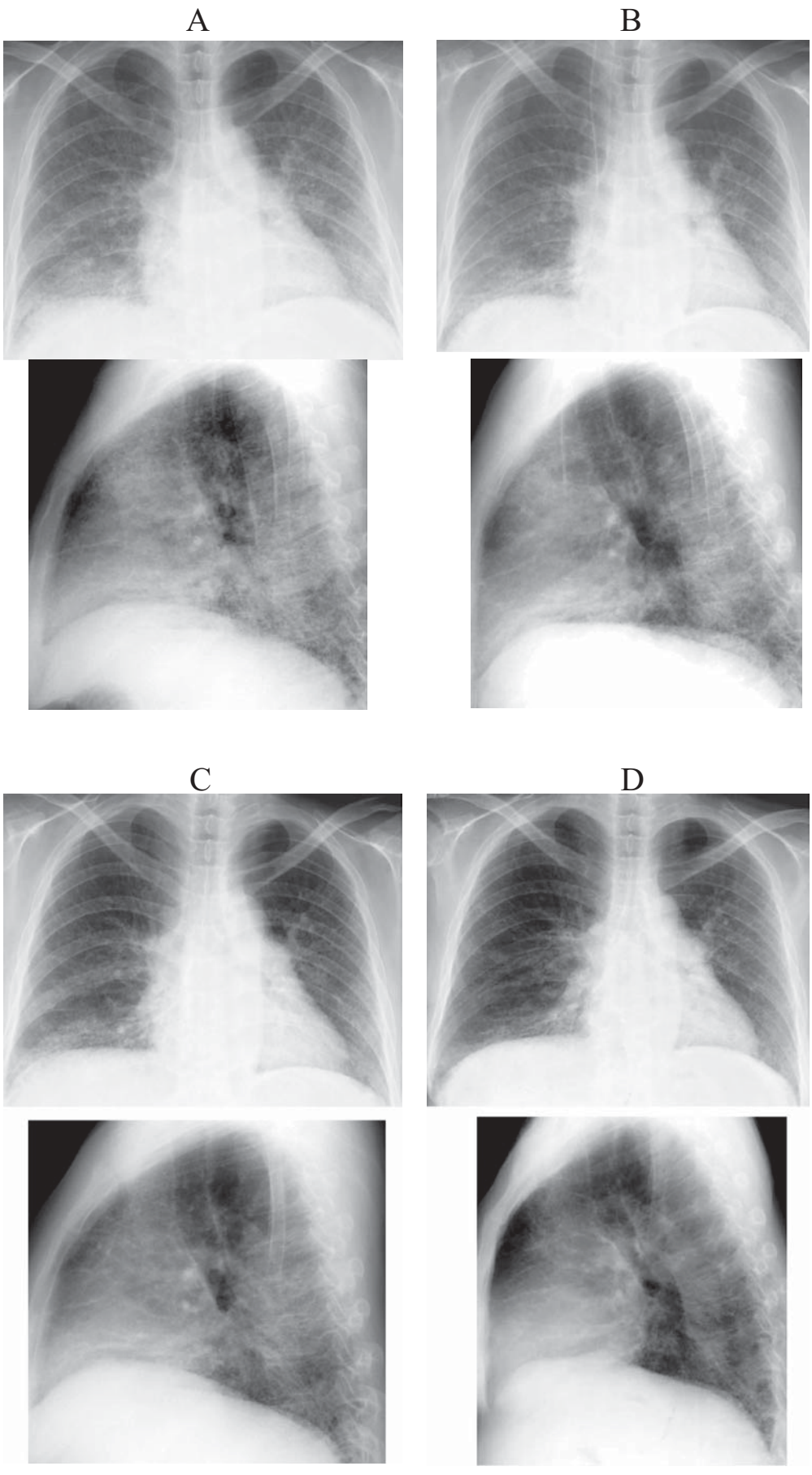

Fig. 2A–D. Chest X-ray findings on admission, before and after UT pulse therapy. **A** Chest X-ray showed ground-glass attenuation in the middle and lower lung zones bilaterally with cardiac enlargement (cardiothoracic ratio, 58%). **B** Three weeks after IVCY and just before initial UT pulse therapy; the ground-glass attenuation in the lungs and cardiac enlargement were almost similar to those noted on admission (compare with Fig. 1A). **C** One month after initial UT pulse therapy; ground-glass attenuation areas were markedly diminished. **D** Six months after the nine courses of UT pulse therapy, the ground-glass attenuation areas almost disappeared and the cardiothoracic ratio diminished to 47%

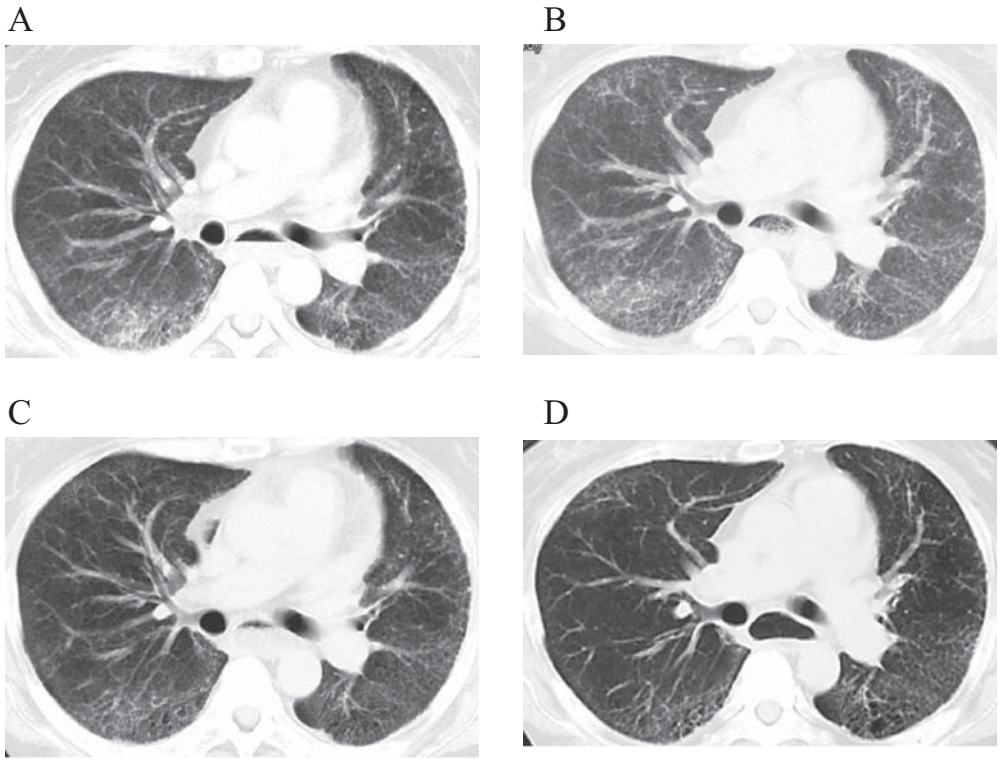

Fig. 3A–D. Chest computed tomographic findings on admission, before and after UT pulse therapies. **A** Chest computed tomography obtained at the level of the bronchus intermedius showed diffuse ground-glass attenuation and irregular linear hyperattenuated areas. **B** Three weeks after IVCY and just before initial UT pulse therapy, irregular hyperattenuated areas in the ventral fields of both lungs were more prominent than those noted on admission (compare with Fig. 2A). **C** One month after initial UT pulse therapy, ground-glass attenuation areas were markedly diminished. **D** Six months after nine courses of UT pulse therapy, the ground-glass attenuation areas almost disappeared although honeycombing of subpleural areas was still noted

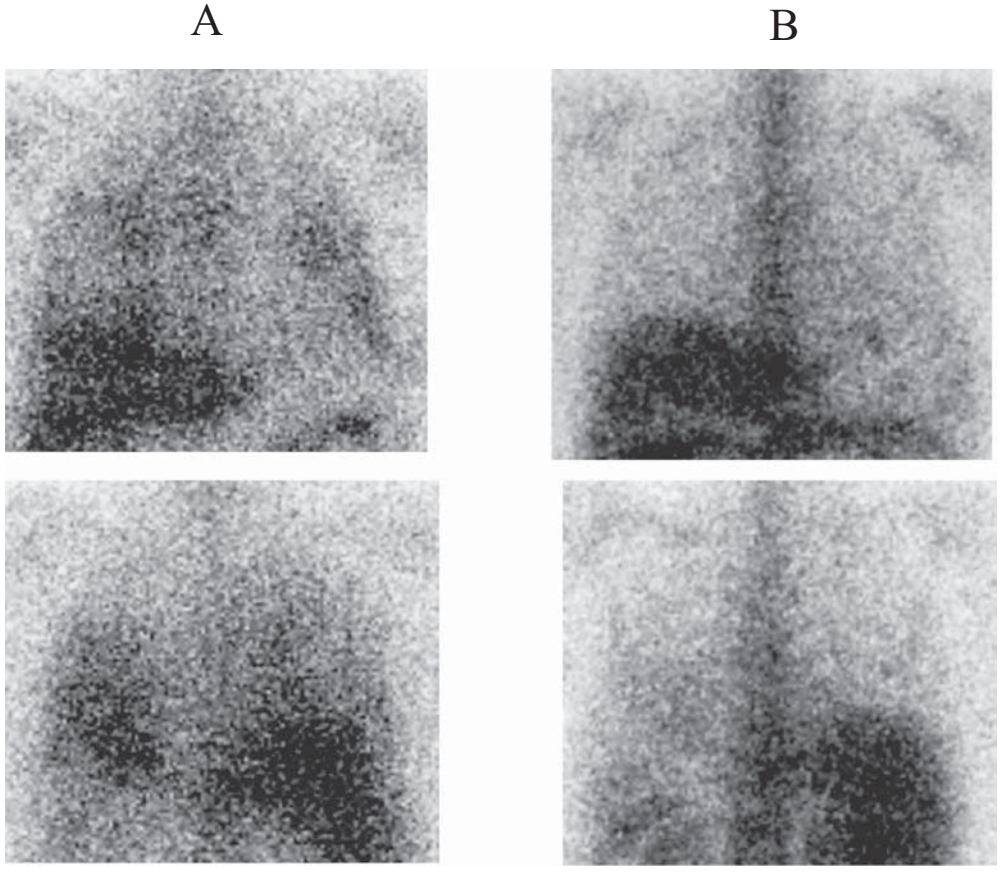

Fig. 4A,B. Gallium scintigrams of the chest before and after UT pulse therapy. **A** On admission; the gallium scintigram revealed marked abnormal isotope accumulation with dorsal predominance in the middle and lower lung zones bilaterally. **B** After 5 courses of UT pulse therapy; the abnormal isotope accumulation in the lungs had almost disappeared

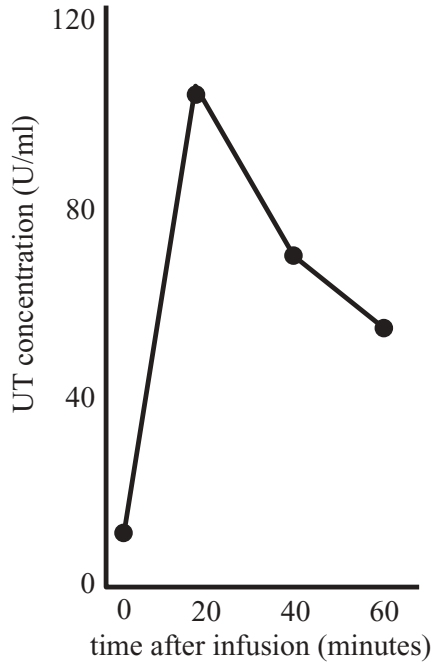

Fig. 5. Kinetics of human urinary trypsin inhibitor (*UT*) concentration in arterial blood after infusion of 3×10^5 units of UT via a catheter inserted into the internal cervical vein. Arterial blood samples were collected from the brachial artery over a period of 60 min and the serum concentrations of UT were measured

have reported that ICAM-1, interleukin-8, and $AaDO_2$ did not significantly change after intravenous UT infusion (1.5×10^5 unit $\times$ 2/day for 5 days).[29] One of the reasons responsible for the failure to establish UT as effective therapy is that the UT blood concentration scarcely reaches an effective level because the half-life of UT is only 40 min.[30] Others have reported that suppression of LPS-induced cytokine production required UT concentrations of 100 units/ml;[31] suppression of oxygen radical required 30 units/ml or more,[31] and suppression of MMP production required 40 unit/ml or more.[19] However, when UT is administered to a peripheral vein, it is impossible to arrive at these levels of UT concentration by conventional infusion of 1.0×10^5 units and, in addition, it is only for a few minutes that UT reaches sufficient concentration even after infusion of 3.0×10^5 units.[32] Our UT pulse therapy overcame this issue by bolus infusion of UT into the central venous catheter, which resulted in sufficient UT concentrations in the pulmonary circulation (Fig. 5) to produce inhibition of cytokines, oxygen radicals, and MMP. Actually, in the present case MMP-9, which has been known to rise more in BOOP,[26] decreased within several hours after UT bolus infusion. The mechanism of action of UT appears to be different from those of conventional immunosuppressive cytotoxic agents, and the present case emphasizes the potential of UT for IP in MCTD patients refractory to conventional therapies.

While the use of UT in more patients with refractory IP is required to confirm our findings, we believe that UT bolus infusion therapy can be potentially used as an alternative therapy for refractory IP, based on its anti-inflammatory and antioxidant effects and lack of serious adverse effects, e.g., bone marrow suppression, organ failure, and opportunistic infection.

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