AN EVALUATION OF FIBRINOLYTIC THERAPY OF PULMONARY THROMBOEMBOLISM USING NON-INVASIVE EXAMINATIONS AND THE RELATIONSHIP OF UROKINASE DOSAGE TO CLINICAL OUTCOME AND COAGULATION-FIBRINOLYTIC CHANGES

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We administered urokinase (UK) 24×10^4 unit (U.) + dextran sulfate (DS) 1,500 or 3,000 mg (UK₂₄), UK 48×10^4 U. + DS 3,000 mg (UK₄₈) and UK 60×10^4 U. (UK₆₀) over 6 hrs daily for 5 consecutive days to 26 patients with pulmonary thromboembolism (PTE). The fibrinolytic effect of UK was evaluated by a combination of clinical and laboratory findings (score), isotopic perfusion defect and A-a DO₂. Prominent recovery was observed in 60% cases of the UK₄₈ and UK₆₀ groups but none in the UK₂₄ group. Fibrin degradation products (FDP) D and D-D increased significantly when α_2 plasmin inhibitor (α_2 PI) decreased to less than 3 mg/dl. These results suggest that fibrinolytic therapy of PTE can be evaluated objectively using non-invasive examinations and UK treatment is successful when 48 or 60×10^4 U. of UK is administered over 6 hrs and a 50% decrease of α_2 PI is obtained.

PULMONARY thromboembolism (PTE) is a life-threatening disease whose incidence has gradually increased in Japan.¹ But the diagnosis of PTE can not be made easily and the fibrinolytic therapy of PTE has not been established. Recently, attempts have been made to diagnose PTE by a combination of non-invasive examinations.¹⁻⁴ We proposed the diagnostic criteria for PTE from an analysis of 54 patients with PTE.^{1,4}

In the present study, we administered urokinase (UK) to 26 patients with PTE and investi-

gated the methods to evaluate objectively the fibrinolytic effect of UK using these criteria. We also examined the relationship of UK dosage to clinical outcome and coagulation-fibrinolytic changes.

MATERIALS AND METHODS

1. Patients: Twenty-six PTE patients (10 males and 16 females, age 52.6 ± 15.4 years, body weight 56.4 ± 5.9 kg, mean $\pm$ S.D.) satisfied the diagnostic criteria for PTE (Table I) and were diagnosed as having PTE. They were classified into 5 types according to the occlusive portions of the pulmonary arteries and the pathophysiological conditions as previously reported.^{1,5} In this study, 9 massive, 4 recurrent, 3 diffuse microembolic, 10 submassive and pulmonary infarction (PI) cases were included (Table II).

Key words:

Fibrinolytic therapy of pulmonary thromboembolism
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TABLE I DIAGNOSTIC CRITERIA FOR PULM. THROMBOEMBOLISM

I. Predisposing factors	(Score)	VI. Laboratory findings	(Score)
1) Cancer	1	1) Ht (≤ 35)	1
2) Phlebitis	1	2) LDH (> 450)	1
3) Heart disease	1	3) GOT (> 40)	2
4) Operation	1	4) GPT (> 35)	2
II. Presenting symptoms		5) Bilirubin (≥ 1.2)	2
1) Cough	1	6) BUN (> 20)	2
2) Hemoptysis	2	7) Leucocytes ($> 8,000$)	3
3) Chest pain	2	8) Plateletes ($< 20 \times 10^4$)	3
4) Dyspnea	2	9) FBG (≤ 200)	1
III. Presenting signs		10) PLG (< 10)	2
1) Syst. hypotension (≤ 100)	1	11) PT (> 13)	2
2) Rales	1	12) FBG (≥ 350)	2
3) Fever ($\geq 37^\circ\text{C}$)	1	13) ATIII (≤ 25)	2
4) Pulse (> 100)	1	14) FDP (≥ 10)	3
5) Hepatomegaly	2	15) pH (> 7.45)	1
6) Respiratory rate (> 16)	3	16) PaCO ₂ (< 40)	2
IV. Chest X-ray abnormalities		17) PaO ₂ (< 65)	4
1) High diaphragm	1	VII. Lung scanning	
2) Plump hilum	1	1) Segmental or lobar perfusion defects	
3) Reticular or nodular shadow	2	2) Ventilation-perfusion mismatch	
4) Pleural effusion	2	VIII. Pulmonary arteriography	
5) Consolidation	2	1) Cut off	
V. ECG findings		2) Filling defects	
1) RBBB	1		
2) S _I Q _{II} T _{III}	1		
3) P _{II}	1		
4) Right axis deviation	2		

Judgement: 1. Lowly probable : more than 15 scores; 2. Highly probable : more than 20 scores;
 3. Definite : 1. + VII 2) and/or VIII 1) or 2)
 2. + VII 2)

2. *Administration of UK*: UK (Mochida Co., Tokyo, Kowa Co., Nagoya, and Green Cross Co., Osaka, 6,000 unit (U.)/vial, respectively) was infused into the patients at doses of 24×10^4 U. with dextran sulfate (DS, Kowa Co.) 1,500 or 3,000 mg (UK₂₄ group, n = 6), 48×10^4 U. with DS 3,000 mg (UK₄₈ group, n = 14) and 60×10^4 U. (UK₆₀ group, n = 6), respectively over 6 hours daily for 5 consecutive days. When hemorrhagic complications were observed (n = 3) or patients died (n = 3), administration of UK was discontinued.

3. *Administration of heparin (Hp)*: In order to prevent the growth and new formation of

thrombi, Hp (Novo Co., Denmark, 25,000 U/ml) $1 \sim 2.5 \times 10^4$ U./day was injected subcutaneously in 2 or 3 installments until at least 14 days after UK therapy and coagulation-fibrinolytic tests became stable. Dosage of Hp was adjusted to keep the activated partial thromboplastin time in the range of 1.5~2 times the normal values. In cases of hemorrhage, Hp injection was also discontinued. No other drugs which can affect the coagulation-fibrinolytic system were administered.

4. *Evaluation of UK therapy*: Before and after the UK therapy, we examined the scores from diagnostic criteria, isotopic perfusion defect

TABLE II LISTS OF THE CASES INVESTIGATED

UK (U)	24×10^4	48×10^4	60×10^4
<i>Effective (n = 12)</i>			
Massive		3	4
Diffuse micro-embolic		1	
Recurrent		2	
Submassive and PI		2	
Total	0	8	4
<i>Days from onset</i>			
		6.0 ± 8.6	14.0 ± 9.1
		8.9 ± 9.2	
<i>Ineffective (n = 14)</i>			
Massive	1	1	
Diffuse micro-embolic	2		
Recurrent		1	1
Submassive and PI	3	4	1
Total	6	6	2
<i>Days from onset</i>			
	13.3 ± 16.2	17.8 ± 13.5	$4^*, 11$
		14.4 ± 13.6	
*: Days from the last episode			(m. $\pm$ S.D.)

(IPD, %) from pulmonary perfusion scintigram by the methods modified from the Urokinase Pulmonary Embolism Trial⁶ and alveolar-arterial oxygen difference (A-a DO₂, torr). Then, we compared the degrees of improvement of these scores (Δ score), IPD (reperfusion area; Δ IPD/IPD pretreatment, RPA) and A-a DO₂ (Δ A-a DO₂). In cases in which fibrin degradation product (FDP) D-D dimer was assayed (n = 8), this parameter was also added to evaluate the effect of UK. And finally, we classified all cases into 3 groups of excellent, moderate and ineffective (I) and the former two were considered as the effective (E) group (Table III).

5. Coagulation-fibrinolytic examinations: Venous blood was withdrawn into one-tenth volume of 3.8% sodium citrate before and after the each administration of UK. Coagulation-fibrinolytic tests were performed by the following methods: fibrinogen (Fbg) by the Tyrosine method (normal values; 150~350 mg/dl), FDP by the latex agglutination test (Teikokuzoki Co., Tokyo; < 5 μ g/ml), FDP-D by the enzyme-linked immunosorbent assay (EIA, Daiichikagaku Co., Tokyo; < 0.68 μ g/ml), FDP D-D dimer via the latex agglutination test (Fujirebio Co., Tokyo; negative), α_2 -plasmin inhibitor (α_2 PI) by a single radial immuno diffusion (SRID; 5~8 mg/dl) and EIA (Teijin Co., Tokyo; 5~7 mg/dl),

TABLE III CRITERIA FOR THE EVALUATION OF UK THERAPY

Parameter ¹⁾	Effective		Ineffective
	Excellent	Moderate	
Score	Score < 15 or $15 \leq \Delta$ Score ²⁾	$15 \leq$ Score < 20 or $10 \leq \Delta$ Score < 15	
Reperfusion area ³⁾	$\geq 50\%$	$30\% \leq$ < 50%	All cases except effective
A-a DO ₂	$30 \leq \Delta$ A-a DO ₂ ²⁾ torr	$20 \leq \Delta$ A-a DO ₂ < 30 torr	
Judgement	1) when satisfied more than 2 items 2) 1 item + D-D dimer (++)	1) when satisfied more than 2 items 2) 1 item + D-D dimer (+) 3) 1 item + (#)	

(#): When all of the 3 expert physicians in pulmonology evaluate the effect of UK therapy

1): values just after UK therapy

2): differences between the values of pre and post UK therapy

3): Ratio of the reperfusion area to the IPD of pretreatment (= Δ IPD/IPD pretreatment)

Fig.1. Changes of score, IPD and A-aDO₂. Fig.2. Changes of α₂PI after UK administration.

Fig.3. Changes of PLG, Fbg, FDP and B β .

Fig.4. Changes of FDP-D.

α_2 PI-plasmin complex (α_2 PI-C): EIA (Teijin Co.; $< 1 \mu$ g/ml), plasminogen (PLG) by the SRID (Behringwerke Co., West Germany; 10~30 mg/dl), B β ₁₅₋₄₂ peptide (B β) by radioimmunoassay (RIA, IMCO Co., Sweden; < 2 pmol/ml).

6. Statistical analysis: Statistical significance was examined according to χ^2 -test and t-test. The values of Fbg, PLG and α_2 PI were expressed as a percentage of pre-treatment levels.

RESULTS

1. Clinical outcome: Twelve of the 26 cases belonged to the E group (3 cases; excellent, 9

cases; moderately effective). They were all in the UK₄₈ and UK₆₀ groups (12/20, 60% cases) and none in the UK₂₄ group ($p < 0.05$). Significant improvement was observed in 7/9 (78%) cases of massive type, and in 2 cases (10%) of submassive and PI type ($p < 0.05$, Table II). Clinical conditions in these cases improved dramatically after the 2nd and 3rd administration of UK. Pre-treatment levels of the score, IPD and A-a DO₂ demonstrated no difference between the E and I groups, but Δ score and RPA revealed significant difference ($p < 0.01$) after the UK therapy between these two groups (Fig. 1). Slight hemorrhagic accidents from the venipunctured sites were observed in 3 cases of the E group.

Fig.5. Changes of FDP D-D and α_2 PI·C. Fig.6. Changes of α_2 PI·C, FDP D and D-D dimer between the cases of α_2 PI more than 3 mg/dl and less than 3 mg/dl.

In these cases, α_2 PI was assayed by EIA. (normal values; 5 ~ 7 mg/dl)

2. Coagulation-fibrinolytic changes: When determined by the SRID method, α_2 PI decreased dose-dependently on UK and revealed 71.7 ± 34.3 , 52.8 ± 8.3 and $34.5 \pm 14.8\%$ of the pre-treatment levels after the 3rd infusion of UK in UK₂₄, UK₄₈ and UK₆₀ groups, respectively. But no significant difference was found between the E and I groups (Fig. 2).

PLG decreased gradually to about 60% of the pre-treatment levels in UK₄₈ and UK₆₀ groups, but remained at about 80% in UK₂₄ group. No difference was found between the levels of PLG

in the E and I groups. Fbg did not show any remarkable changes in UK₂₄ group but decreased to about 70% of the pre-injected levels in UK₄₈ and UK₆₀ groups. In the E group, FDP was elevated at $28.3 \pm 23.1 \mu\text{g/ml}$ before the UK treatment and increased rapidly after the UK infusion. However, in the I group, it was at $8.6 \pm 5.6 \mu\text{g/ml}$ before the UK treatment. B β showed prominent elevation after the each administration of UK in the E group (Fig. 3).

FDP D, D-D dimer and α_2 PI·C were examined in 8 cases, of which 4 cases belonged to the E

group and 4 cases to the I group. In these cases, α_2 PI was also assayed by EIA. FDP D increased significantly from the 1st to the 3rd infusion of UK in the E group and FDP D/FDP ratio showed high levels. But it remained at low levels in the I group (Fig. 4). When the positive agglutination titre was expressed following the method of Elms,⁷ FDP D-D dimer increased significantly in the E group, but no remarkable elevation was observed in the I group. α_2 PI-C increased prominently after the each injection of UK in both groups (Fig. 5). FDP D, D-D dimer and α_2 PI-C demonstrated significant increase when α_2 PI measured by EIA was less than 3 mg/dl (Fig. 6).

DISCUSSION

Pulmonary angiography is the most reliable procedure for diagnosing PTE although it is not easily performed because of the risk to the patients.^{8,9} Lung scanning is a non-invasive examination, but it does not always provide a definite diagnosis because IPD is observed in many other pulmonary diseases besides PTE; it only estimates the capillary blood flow and does not demonstrate the existence of thrombi attached to the large vessel walls.^{10,11} On the other hand, the score is composed of many clinical and laboratory findings which are not specific for PTE and A-a DO₂ is affected by various factors related to pulmonary function. However, we reported that score, IPD and A-a DO₂ correlated well with Miller's angiographic index¹² and the diagnostic sensitivity of the combination of these non-invasive tests for PTE was high.⁴ In the present study, we used these examinations to evaluate fibrinolytic therapy of PTE and demonstrated that the fibrinolytic effect of UK could be estimated objectively in numeral figures. So, it should be considered hereafter that fibrinolytic effect is compared on the basis of common criteria like the present ones.

Although PTE has been thought to be a good indication of fibrinolytic therapy and large-scale studies have been carried out,^{6,13} standard methods of UK therapy on PTE have not been established. Previously, we reported that clot lysis was observed experimentally in the cuvette of the thromboelastogram when UK was incorporated into the plasma at levels of more than 100 U/ml.¹⁴ This means that more than 24×10^4 U. of UK should be administered to the patients according to the patients' plasma

volume. However, there are many other still unresolved yet important factors which must be taken into account in vivo.^{1,14,15} On the other hand, we¹⁶ and Tangen et al.¹⁷ have previously reported that DS potentiates the fibrinolytic activity. Therefore, in this study, we administered three different doses of UK with or without DS over 6 hours to 26 patients. These doses were relatively small compared with previously reported ones, but we obtained good results similar to the previous studies in 78% patients with massive PTE and in 60% cases of UK₄₈ and UK₆₀ groups.^{6,13} Hemorrhagic episodes at the venipunctured site were observed in 3 (12%) cases, but the rate was lower when compared with the other studies.^{6,13}

In coagulation-fibrinolytic examinations, the fibrinolytic effect was reflected by the levels of FDP D and D-D dimer. Prominent elevation of these factors was found to occur from the 1st to the 3rd infusions of UK, accompanied by the dramatic improvement of clinical symptoms and an early increase of FDP and B β in the E group. FDP D and D-D dimer increased significantly when α_2 PI was assayed by EIA and revealed less than 3 mg/dl (almost 50% of the normal levels). However, no significant difference was found in the levels of α_2 PI measured by SRID between the E and I groups and both showed higher levels than the values obtained by EIA. These results demonstrated that α_2 PI-C was determined simultaneously with non-complexed α_2 PI by SRID methods. So, it is suggested that EIA is more suitable. In UK₂₄ group, coagulation-fibrinolytic changes were minute and the decrease of α_2 PI was significantly lower than the changes in UK₄₈ and UK₆₀ groups. Therefore, it is not surprising that we obtained no remarkable fibrinolytic activities in UK₂₄ group.

From these results, we conclude that the fibrinolytic effect of UK on PTE can be evaluated clinically by the combination of scores, IPD and A-a DO₂ and the determination of FDP D and D-D dimer provides direct evidence of fibrinolysis in vivo. The administration of 48×10^4 U. or 60×10^4 U. of UK over 6 hrs and approximately a 50% decrease of α_2 PI are necessary for successful UK treatment.

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