Role of Dextran Sulfate in Urokinase Therapy and Evaluation of the Effects by Estimation of Plasmin Inhibitor, Fibrinogenolytic Degradation Products, and Fibrinolytic Degradation Products

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SUMMARY

The present study was concerned with changes in APTT and coagulation-fibrinolytic inhibitors in 2 groups of patients receiving either UK with heparin or UK with heparin and dextran sulfate (DS).

APTT just after UK treatment showed a significant prolongation in patients receiving combination treatment with DS, but contrarily a shortening in patients receiving those without DS. This result indicated the usefulness of DS combination in UK treatment.

There was a marked difference between the responses of α_2-PI and antiplasmin to the treatment, presumably as a result of immune complex formation of the α_2-PI with plasmin. However, no marked difference in α_2-PI and AT-III existed both just after and at 24 hours after UK treatment in patients receiving UK (240,000 units) with both heparin and DS and those receiving UK (480,000 units) with heparin. Those results suggest that the dosage of UK can be reduced by combination of DS.

Successful UK treatment in clinical cases resulted in a decrease of α_2-PI and an increase of fibrinogenolytic or fibrinolytic degradation products. For these reasons, effective UK treatment appears to require combination with DS and several treatment days under the condition with a decrease of α_2-PI (50%) and fibrinogenolysis with fibrinolysis, which are followed by administrations of UK.

Additional Indexing Words:

Activated partial thromboplastin time (APTT) Antithrombin III (AT-III) Urokinase (UK) Dextran sulfate (DS) α_2-Plasmin inhibitor (α_2-PI) Fibrinolysis Fibrinogenolysis

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UROKINASE (UK) does not have antigenic properities, but it does have the activity as a plasminogen activator, for which reason it is widely used as a thrombolytic agent by intravenous injection. However, many problems remain to be solved concerning thrombolytic therapy in general. The UK dosage employed in Japan, for instance, has been gradually increased in recent years, but, is still far less than that used in USA.[1] The dosage and criteria for rational use of UK remain unsolved. To answer the question satisfactorily one needs to approach it from 2 different aspects: First, what effects do UK have on coagulation-fibrinolytic factors in the blood stream, and what are the conditions of these factors at which the greatest effect of UK can be achieved? Secondly, are the characteristics of the thrombus in requirement such as to allow UK to have effect? As regards the first question, it involves a number of subjects to be investigated, including shortening of activated partial thromboplastin time (APTT) following administration of UK, changes in levels of inhibitors in the circulation following UK therapy, and so forth. In the study presented here an attempt was made to compare mainly such changes in the blood level of inhibitors in 2 groups of patients receiving either UK with heparin or UK with heparin and dextran sulfate (DS) and to estimate in coagulation-fibrinolytic factors in patients receiving UK with DS for 3 days. According to the analysis of its results the further attempt was made to determine the coagulation-fibrinolytic indexes for assessment of UK therapy.

Subjects and Methods

The subjects were 12 in-patients of the 1st Department of Medicine, Hokkaido University Hospital, receiving thrombolytic therapy (for pulmonary embolism, arterial thrombosis of the leg, or deep vein thrombosis). Prior to the administration of UK, 25,000 units of heparin were injected subcutaneously to 8 patients. After the heparin effect had appeared, 4 of the patients were administrated 480,000 units of UK and another 4 patients 240,000 units of UK and 3,000 mg of DS. The time required for intravenous infusion of UK ranged from 4 to 12 hours, 50,000 units invariably being infused in the first 1 hour. The remaining patients received 480,000 units of UK and 3,000 mg of DS daily for 3 consecutive days.

Blood samples were collected before, just after, 24 hours and 48 hours after UK infusion, and then submitted to measurement of the following parameters: α_1-antitrypsin (α_1-AT), α_2-macroglobulin (α_2-M), C_1 inactivator (C_1-INA), and antithrombin III (AT-III) [using M-partigen, Behringwerke], α_2-plasmin inhibitor (α_2-PI)[8] (single radial immunodiffusion), AT-III activity

and antiplasmin activity [chromogenic substrate assay, KABI], APTT (Dade & General Diagnostic), fibrinogen (modified tyrosine method), plasminogen (M-partigen), whole plasmin activity (WPA) (standard plate method), fibrin degradation products (FDP) (Teikoku-Zoki), heparin concentration (KABI) [chromogenic substrate assay], and fibrinopeptide A (FPA) (IMMUCO).

Normal values for these parameters from 10 healthy adults were estimated as follows: α_1-AT 210–500 mg/100 ml, α_2-M 220–380 mg/100 ml, C_1-INA 15–35 mg/100 ml, AT-III 28–33 mg/100 ml, α_2-PI 80–120% (100%: 5.8 mg/100 ml), antiplasmin activity 80–120%, AT-III activity 80–120%, APTT 27–35 sec and 38–45 sec, fibrinogen 150–350 mg/100 ml, plasminogen 10–15 mg/100 ml, WPA 1,400–1,600 mm^2, FDP<5 μg/ml, effective blood heparin concentration 0.2–1.2 U/ml, FPA<2 ng/ml.

Results

1) α_1-AT and α_2-M

In the 8 patients, α_1-AT and α_2-M were 296 ± 30 mg/100 ml (mean$\pm$S.E.) and 170 ± 17 mg/100 ml before UK treatment, 270 ± 28 mg/100 ml and 180 ± 13 mg/100 ml just after UK treatment, and 311 ± 4 mg/100 ml and 189 ± 13 mg/100 ml at 24 hours after UK treatment. α_1-AT levels remained within the normal range during UK treatment, while α_2-M levels were lower than normal but no significant change before and after UK treatment.

2) C_1-INA

In the 8 patients, C_1-INA were 29.3 ± 2.9 mg/100 ml before UK treatment, 24.8 ± 2.2 mg/100 ml just after UK treatment and 28 ± 3.3 mg/100 ml at 24 hours after UK treatment. C_1-INA levels remained within the normal range during treatment with UK, although they were somewhat lower both just after and 24 hours after treatment with UK than before UK treatment. No significant difference was present between patients receiving UK with heparin and those receiving UK with heparin and DS.

3) AT-III

In the 8 patients, AT-III were 31 ± 2.5 mg/100 ml before UK treatment, 25.2 ± 2.0 mg/100 ml just after UK treatment, 25.4 ± 2.4 mg/100 ml at 24 hours and 27.5 ± 2.0 mg/100 ml at 48 hours after UK treatment. AT-III levels tended to be significantly lower both just after and 24 hours after treatment with UK than before treatment, and returned to the initial levels at 48 hours. The decrease in AT-III levels tended to be somewhat less marked at 24 hours in patients receiving UK with heparin than in those receiving UK with heparin and DS. In a 56-year-old patient, AT-III activity just after UK treatment as determined by the chromogenic substrate assay was ap-

proximately 35% lower than that determined by the immunoassay.

4) α_2-PI

In the 8 patients α_2-PI were 100.0±3.2% before UK treatment, 78.0±4.0% just after UK treatment, 78.0±4.0% at 24 hours and 86.5±7.0% at 48 hours after UK treatment. α_2-PI levels tended to be much lower both just after and 24 hours after UK treatment than before UK treatment, and returned to the initial levels at 48 hours. No marked difference existed in this regard between patients receiving UK with both heparin and DS and those receiving UK with heparin, although the latter group showed a stronger tendency for α_2-PI levels to return to the initial values at 48 hours. A significant difference existed between α_2-PI as determined by immunoassay and antiplasmin activity by chromogenic substrate assay just after UK treatment, when the former was 23% and the latter 53% lower than before treatment (Fig. 1).

5) APTT

In patients receiving UK with heparin or with heparin and DS, APTT were −53±12 sec or 108±74 sec just after UK treatment, 18±35 sec or 52±43 sec at 24 hours, and 11±15 sec or 7.0±26 sec at 48 hours after UK treatment, as compared to 0±62 sec or 0±17 sec before UK treatment. Just after treatment with UK, APTT tended to be shorter in patients receiving

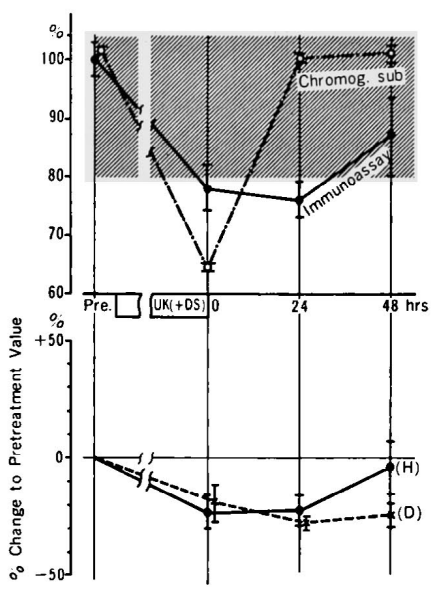

Fig. 1. Upper figure: Comparison of immunological and enzymatical values in α_2-PI. Lower figure: Changes in α_2-PI during UK treatment with heparin and with heparin and DS. UK dosages: 480,000 units in the former, 240,000 units in the latter. (H)=UK+Heparin; (D)=UK+Heparin+DS.

UK with heparin than before UK treatment, but contrarily longer in those receiving UK with heparin and DS than before UK treatment (Fig. 2).

6) *A case in the 4 patients treated with 480,000 units of UK and 3,000 mg of DS for 3 consecutive days.*

The patient was a 60-year-old man presenting symptoms and signs of recurrent pulmonary thromboembolism (Table I). After UK treatment, per-

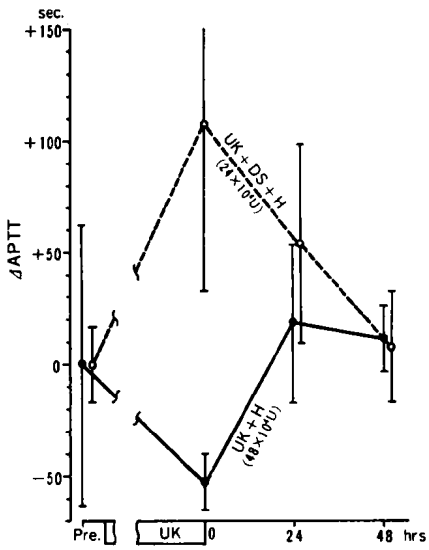

Fig. 2. Comparison of APTT during UK treatment with heparin and with heparin and DS.

Table I. Changes of Coagulation-Fibrinolysis-Thrombocytic Factors in a Patient with UK Treatment for 3 Days

	1st day				2nd day		3rd day		
	hours after infusion of urokinase+DS								
	before	1	3	6	before	6	before	6	24
Fbg (150-350 mg/100 ml)	222.0	244.0	247.0	215.0	186.0	187.0	112.0	100.0	92.0
AT III (28-32 mg/100 ml)	21.1	21.1	21.1	19.8	19.8	21.1	18.5	19.8	19.8
FPA (<2 ng/ml)	20.0	3.0	8.4	7.2	12.4	8.7	4.3	10.0	2.2
Plg (10-30 mg/100 ml)	9.8	9.8	8.6	6.9	7.5	5.9	6.4	5.9	4.6
α_2PI (4.5-6.7 mg/100 ml)	4.4	3.9	3.2	2.5	2.6	1.8	2.4	1.9	2.6
FDP (<5 μg/ml)	5.0	5.0	20.0	20.0	5.0	40.0	10.0	20.0	5.0
βTG (11.1-42.7 ng/ml)	166.0	64.0		102.0	155.0	126.0			
PF_4 (2.0-16.4 ng/ml)	29.0	21.4		24.6	32.0	19.4	32.3	90.5	50.6

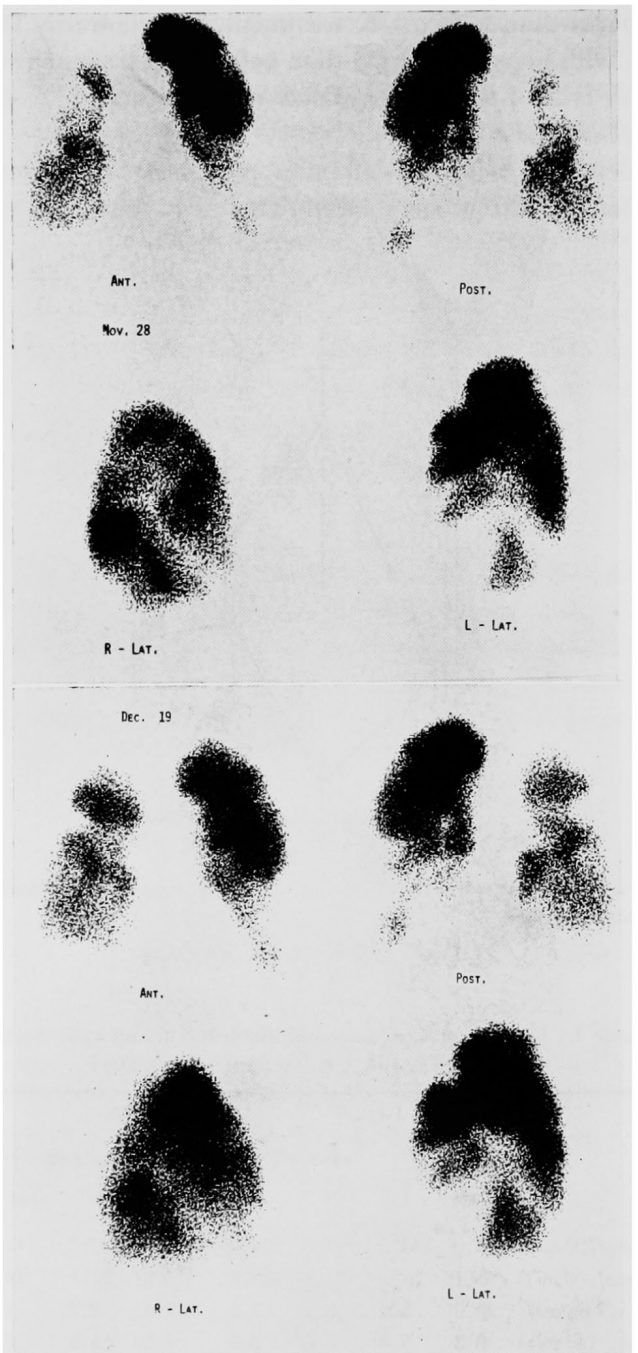

Fig. 3. Perfusion lung scan in a patient with recurrent pulmonary thrombo-embolism. Upper figure: Perfusion scan before UK treatment. Lower figure: Perfusion scan after UK treatment. Perfusion defects were reduced in size after UK treatment.

fusion lung scan revealed a certain reduction of the defect areas (Fig. 3).

Twenty ng/ml of FPA before UK treatment indicated the presence of pulmonary thromboembolism with intravascular coagulation. α_2-PI level were reduced by about 40% of the initial value just after the treatment with UK and DS on the first day, and further reduced by about 60% immediately after the treatment on the second and third day. Plasminogen levels decreased by about 30–40% of the initial value just after the treatment with UK and DS on the first, second, and third day. Fibrinogen level remained as high as before UK treatment with DS on the first day, then reduced by about 15% on the second day and further reduced by about 60% on the third day.

FDP level rose to 20–40 μg/ml just after the treatment with UK and DS on the first, second, and third day. FDP immunoprecipitates made from plasma using antifibrinogen rabbit serum were analyzed by SDS-polyacrylamide gel electrophoresis. A band, which derived from plasma before and after treatment with UK, migrated with the same mobility as D-dimer.

Discussion

The present study was concerned with changes of coagulation-fibrinolytic factors and their inhibitors during UK therapy. No marked change was observed in the levels of α_1-AT or α_2-M. Of interest is the fact that C_1-INA tended to decrease slightly within normal range after treatment with UK, a similar pattern was observed for prekallikren and factor XII. Such a change in factor XII may be associated with shortening of APTT for which several causes have been suggested.[2)-4)] Shortening of APTT during UK treatment may indicate an activation of the coagulation system and a formation of new thrombi. Combination of DS to UK treatment resulted in inhibition of the shortening or APTT. The usefulness of DS combination in UK therapy, therefore, is suggested.

AT-III activity tended to be lower than normal during UK treatment. The decrease of AT-III was more marked when the activity was determined by chromogenic substrate assay than when determined by immunoassay, the difference being approximately 30%. AT-III inhibits thrombin and factor XIIa, XIa, and Xa,[5)] and the inhibitory effect is greatly affected by combination of heparin or DS with UK. The difference just cited is probably explained by an assumption that AT-III is no longer enzymatically active even though still immunologically active, having complex formed after UK treatment.

α_2-PI[8)] level was also reduced after treatment with UK (approximately

by 23% with treatment for a day and by 40–60% with treatment for 3 days). As reported previously,[6),7)] effect of UK cannot be achieved unless UK is administered in an adequate amount, which makes α_2-PI or plasmin inhibitor activity reduced to below 50% of their normal levels. α_2-PI level also tended to fall just after and 24 hours after treatment with 480,000 units of UK and heparin or with 240,000 units of UK, heparin and DS. These results suggest that the dosage of UK can be reduced by combination with DS. There was a difference between α_2-PI level as determined by immunoassay and that by chromogenic substrate assay (a difference of 20% in patients receiving UK for a day and 15–40% in patients receiving UK for 3 days). The difference was interpreted to be resulted from formation of an immune complex with plasmin inhibitor and plasmin.

The fact that there was a decreasing plasminogen level in the patients receiving UK for 3 days underlines the importance of checking plasminogen frequently during a course of UK therapy if satisfactory UK effect is to be achieved. Successful treatment with UK resulted in fibrinogenolysis with fibrinolysis, suggesting manifestation of UK effect. Such a pattern of an increase in FgDP or FDP is probably related with the decrease in α_2-PI and hence fibrinogenolysis or lysis of thrombus. There was also a marked decrease of fibrinogen probably originated from fibrinogenolysis following treatment with UK for 3 days. It is not always easy to differentiate by SDS electrophoresis whether UK treatment was effective for fibrinolysis or not, even if a band migrated with the same mobility as D-dimer had been observed.

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