Effect of Urokinase on Fibrinolysis and Fibrinogenolysis

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Summary The thrombolytic (fibrinolytic) effect of the urinary plasminogen activator, urokinase (UK), was compared with its fibrinogenolytic effect, using an artificial circulating system, and in in vivo pulmonary embolus and femoral vein thrombosis models. The thrombus lysing efficiency (fibrinolysis/fibrinogenolysis) was, in all cases, below 1.00, indicating that fibrinogenolysis is a primary function of UK while fibrinolysis is a secondary one.

Key Words: fibrinolysis, fibrinogenolysis, urokinase.

In thrombus-containing vessels, the blood flow is decreased or ceases, depending on the degree of obstruction of the vessels. The function of thrombus-containing organs is thus more or less disturbed. In order to avoid functional disturbance, the thrombus must be lysed as soon as possible and circulation re-established.

The physiological role of the blood fibrinolytic system is to remove intravascular fibrin, which is a main structural protein of thrombi and which disturbs the flow of blood (Astrup, 1978). Blood fibrinolytic activity can be enhanced through the administration of plasminogen activator (Colfen, 1980). At present, the urinary plasminogen activator, urokinase (UK), is one of the agents which is known to be able to activate the blood fibrinolytic system and lyse thrombi (Matsuo et al., 1980). UK is probably present in the urine of all mammals, and clinical UK preparations have been used for thrombolytic therapy. However, the dose of UK has not been standardized yet: doses range widely from small (e.g., 10,000 IU) to very large (several million IU) (see reviews by Duckert, 1978 and Matsuo et al., 1979). When the blood fibrinolytic system is activated by UK, plasmin is produced in the circulation and digests fibrin as well as circulating fibrinogen. Fibrin formation enhances the activation of Glu-plasminogen by UK, but not that of Lys-plasminogen (Takada and Takada, 1980). UK is
generally administered after clot (fibrin) formation in order to lyse fibrin (TILSNER and LENAU, 1980; MANNUCCI and D'ANGELO, 1982). Digestion of fibrinogen (fibrinogenolysis) may lead to a hemorrhagic tendency, which is a contraindication against the administration of fibrinolytic enzyme. Discrimination of fibrinogenolysis from fibrinolysis is therefore important in the management of thrombolysis (fibrinolysis). In the present study, the effects of UK in terms of such discrimination were observed.

Radiolabelled thrombi which were used in the present study were prepared by the following procedures: Using purified and labelled fibrinogen, radiolabelled thrombi were produced with fresh human plasma, purified thrombin and CaCl₂ (MATSUO et al., 1981a). The thrombus-lysing efficiency was calculated from the ratio of the degree of fibrinolysis to that of fibrinogenolysis. In the case where the thrombus-lysing efficiency exceeded 1.00, fibrinolysis was considered predominant over fibrinogenolysis, and vice versa.

For in vitro experiments: An artificial circulation system was set up with two chambers connected by tubes, and 50 ml of fresh frozen blood bank plasma was circulated with a pump (MATSUO et al., 1981a). A radiolabelled thrombus was allowed to sink in one chamber, and UK was administered to the other chamber employing concomitant administration of one shot and continuous infusion. With this system, the degree of fibrinolysis (thrombolysis) as well as of fibrinogenolysis could be estimated simultaneously: the degree of thrombolysis was calculated from the radioactivity released in the circulating plasma, and the degree of fibrinogenolysis was calculated from the fibrinogen content of the circulating plasma.

The effect of UK on thrombolysis was investigated using various concentrations of UK (Fig. 1). At low UK concentrations below 20 IU/ml, the degree of thrombolysis was not very different from that following saline injection. As the UK concentration rose, the degree of thrombolysis showed a sharp rise, although at UK concentrations above 260 IU/ml the degree of thrombolysis remained rather steady. The \( L_{50} \) (50% lysis of thrombus) value was about 340 IU/ml. At a UK concentration of 100 IU/ml, the degree of thrombolysis was 16% and that of fibrinogenolysis 29%, so that the thrombus-lysing efficiency was 0.55. At a higher UK concentration (260 IU/ml), the degree of thrombolysis increased to 66%, but the circulating fibrinogen was completely digested, so that the thrombus-lysing efficiency was 0.66.

In the in vivo experiments, the degree of fibrinolysis (thrombolysis) was compared with that of fibrinogenolysis at the end of the experiment. The degree of fibrinogenolysis was calculated from the fibrinogen content of the plasma before and after the experiment.

(A) Rabbit pulmonary embolus model (MATSUO et al., 1981b): A radioactive thrombus produced as described above was injected with physiological saline into the jugular vein of a rabbit, and an experimental pulmonary embolus
UROKINASE AND FIBRINOLYSIS

Fig. 1. Relation between urokinase concentration and lysis of thrombus. The 50% lysis of thrombus obtained on the graph was about 340 IU/ml of plasma.

was produced. The lysis of the thrombus was estimated on the basis of the radioactivity difference before and after the experiment. The degree of thrombolysis following administration of 100,000 IU was the same as that following saline administration. Administration of 1,000,000 IU caused thrombolysis (12%), which was accompanied by marked fibrinogenolysis (42%). The thrombus-lysing efficiency was thus 0.28.

(B) Dog femoral vein thrombosis model (KORNINGER et al., 1982): A radioactive thrombus was produced by injecting radioactive fibrinogen, thrombin and CaCl₂ into a segment of the femoral vein. The lysis of the thrombus was estimated on the basis of the difference between the injected radioactivity and the radioactivity recovered from the thrombus. The degree of thrombolysis following administration of 100,000 IU did not differ from that following saline administration. On administration of 1,000,000 IU, remarkable thrombolysis (41%) was observed which was accompanied by extensive fibrinogenolysis (100%). The thrombus-lysing efficiency was thus 0.41.

Circulatory disturbance due to thrombosis in the brain or heart may lead to a serious clinical condition, and that in the lung, legs or other organs may also give rise to functional loss. Re-establishment of circulation is thus a major aim for avoiding functional disturbance. Since fibrin is a major structural protein of thrombi, urinary plasminogen activator, urokinase (UK), is in popular use for activation of the blood fibrinolytic system and lysing thrombi. However, there is as yet no general consensus regarding the mode of UK administration or the dosage of UK to be employed.

The conservative approach was that small dosages of UK were thought to be sufficient to lyse a thrombus. However, the plasma UK concentration after administration of a small dosage of UK, as calculated using compartmental
analysis, was found to be too low to lyse the thrombus (MATSUO et al., 1976; MATSUO and MIHARA, 1977). Since such a theoretical approach to thrombolysis has been accepted, the dosage of UK used has been progressively increasing, a high plasma UK concentration thus being attained. However, a high UK concentration is accompanied by fibrinogenolysis as well as fibrinolysis, and the thrombus-lysing efficiency (fibrinolysis/fibrinogenolysis ratio) was found to be below 1.00, i.e., the magnitude of fibrinogenolysis was always larger than that of fibrinolysis. In other words, fibrinolysis is not the primary effect of UK administration, but a secondary one: fibrinogenolysis represents the primary effect.

At present, UK is one of the choices for the treatment of thromboembolism. However, it cannot be regarded as either adequately safe (causing fibrinogenolysis) or effective (thrombus-lysing efficiency below 1.00). Therefore, new types of thrombolytic enzymes with a high affinity for fibrin and which cause only fibrinolysis, must be sought (MATSUO, 1981).

REFERENCES


