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A MECHANISM FOR THE POTENTIATING EFFECT OF UROKINASE (UK) OR TISSUE PLASMINOGEN ACTIVATOR (t-PA) ON CLOT LYSIS BY PRO-UROKINASE (PRO-UK). V. Gurewich, J. Black and R. Pannell. Vascular Laboratory, St. Elizabeth's Hospital, Boston, MA, USA.

Pro-UK has been shown to selectively activate fibrin-bound plasminogen (Blood 67:1215, '86). Synergism with t-PA has been postulated to be related to t-PA's rapid induction of plasmin generation which exposes new plasminogen binding sites on fibrin (JBC Harpel et al 260:4432, '85). As a result, more substrate is made available for pro-UK, thereby attenuating its lag phase and greatly potentiating its fibrinolytic effect (Thrombos. Res. 44:217, '86). This hypothesis was tested with UK, which has a rapid onset of action similar to t-PA. In vitro lysis (^{125}I-clots suspended in citrate plasma), by UK (0.05-0.2 μg/ml) plus pro-UK (0.8-0.95 μg/ml) was compared with that of UK or pro-UK alone. Marked potentiation consistent with synergism by UK was found. For example, 0.1 μg/ml UK plus 0.9 μg/ml pro-UK induced clot lysis equivalent to that of 1.25 μg/ml pro-UK or 1.50 μg/ml UK alone using either 50% or 100% clot lysis as end points. Similar findings were obtained when clots were exposed to these concentrations of UK and pro-UK sequentially instead of simultaneously. For this, a clot suspended in plasma was incubated (37°C) for 1 h. with UK, then removed and suspended in fresh plasma (3 ml) for 1 h. after which pro-UK was added. In another set of experiments, a radiolabeled clot was incubated in buffer containing plasmin (2 μg/ml) for 20 min. (<1% lysis), then transferred to plasma and incubated for 30 min., after which pro-UK (0.8-1.0 μg/ml) or t-PA (0.04 μg/ml) was added. Plasmin pretreatment of clots caused marked potentiation of lysis by pro-UK but did not potentiate t-PA. This potentiation of clot lysis by plasmin pretreatment was nullified by treatment of clots with carboxypeptidase-B, to remove lysine residues.

It was concluded that UK potentiates pro-UK induced clot lysis by exposing plasminogen binding sites on fibrin according to the mechanism postulated above for t-PA. The potentiating effect of UK on pro-UK appears to be analogous to that of t-PA and may also be defined as being synergistic.

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SYNERGY OF TRANEXAMIC ACID AND FIBRIN IN THE ENHANCEMENT OF THE ACTIVATION OF GLU-PLASMINOGEN BY UROKINASE. Y. Sugawara, Y. Takada and A. Takada. Department of Physiology, Hamamatsu University, School of Medicine, Hamamatsu-shi, Shizuoka, Japan

The activation rate of Glu-plasminogen (Glu-plg) by urokinase (UK) was enhanced in the presence of either tranexamic acid or fibrin with increase in catalytic rate constant (k_{cat}) without change in Km. The values of k_{cat} increased almost linearly up to 0.5 mM of tranexamic acid concentration and reached a plateau. On the other hand, the presence of increasing concentrations of fibrin resulted in almost hyperbolic increase in k_{cat} values, reaching the plateau level at 0.04 μM. The values of k_{cat} increased three fold at 0.5 mM of tranexamic acid and at 0.04 μM of fibrin. Fibrinogen was less effective and k_{cat} increased two fold in the presence of 1 μM of fibrinogen. Similar kinetic parameters suggest that basic mechanisms underlying the enhanced activation of Glu-plg by UK may be similar between in the presence of tranexamic acid and fibrin. Possibly conformational changes of Glu-plg upon interaction of its lysine binding sites (LBS) with tranexamic acid or fibrin may be reasons for the enhanced activation. The addition of fibrin to 1 mM tranexamic acid resulted in further increase in k_{cat} of the UK activation of Glu-plg. On the other hand, the addition of tranexamic acid to 0.1 μM of fibrin further increased k_{cat} of the UK activation of Glu-plg. Thus synergy was observed in the activation of Glu-plg by UK between tranexamic acid and fibrin. Fibrin binding site on kringle 5 of Glu-plg may be involved in further increase in the activation rate of Glu-plg by UK in the presence of both fibrin and tranexamic acid in comparison to that in the presence of tranexamic acid alone. Possibly, Glu-plg bound with both tranexamic acid and fibrin (at kringle 5) may be most effectively activated by UK. From the concentrations of fibrin to show 50 % changes of k_{cat}, it is also suggested that two molecules of Glu-plg should bind to one molecule of fibrin monomer.

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ACTIVATION PATHWAYS OF PLASMINOGEN BY UROKINASE AND TISSUE PLASMINOGEN ACTIVATOR IN THE PLASMA OR CLOTTED PLASMA: COMAPRISON WITH PURIFIED SYSTEMS. Y. Makino, M. Yoshida, Y. Takada A. Takada. Department of Physiology, Hamamatsu University, Sch. Med., Hamamatsu-shi, Shizuoka, Japan

The activation pathways of a native plasminogen (Glu-plg) were studied in the plasma and purified systems. When purified Glu-plg was activated by urokinase (UK) or tissue plasminogen activator (t-PA), the activation of Glu-plg proceeded very slowly in the presence of fibrinogen, but rapidly in the presence of non cross-linked or cross-linked fibrin. Little formation of modified plg (Lys-plg) was observed in the presence of fibrinogen, but significant amounts of Lys-plg were formed in the presence of fibrin clot. The addition of $\alpha_2$antiplasmin (α_2AP) to the mixture of Glu-plg, UK or t-PA, and fibrinogen or fibrin resulted in little formation of Lys-plg in the presence of fibrinogen but significant formation of Lys-plg in the presence of non cross-linked or cross linked fibrin. Alpha$_2$antiplasmin did not prevent the formation of Lys-plg from Glu-plg by plasmin in the presence of fibrin regardless of possible cross-linkage of α_2AP to fibrin. In contrast to purified system, the activation of Glu-plg by UK or t-PA in the presence of plasma clot resulted in little formation of Lys plg The addition of thrombin and Ca^{++} to the plasma activated by UK or t-PA resulted in little formation of Lys-plg. These results suggest that Glu-plg was mainly directly activated by UK or t-PA to Glu-plasmin (then Lys-plasmin) in the plasma or plasma clot (non cross-linked or cross-linked), and that some of Glu-plg was converted to Lys-plg by preformed plasmin after UK or t-PA induced activation of Glu-plg in the presence of fibrin. It should be stressed that not all the molecules of Glu-plg were converted to Lys-plg in the presence of fibrin before their activation by activators. Some amounts of Glu-plg were present throughout the activation process in its native form.

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AMINO ACID CHANGES AT LYS-158 THAT ALTER THE SENSITIVITY OF SCU-PA TO CLEAVAGE BY PLASMIN. G.F. VOVIS, J. MAO, R. BROEZE, D. ABERCROMBIE, P. PUMA, K. HSAIO, S. ALMEDA. Department of Molecular Genetics and Biochemistry, Collaborative Research, Inc., Two Oak Park, Bedford, MA, U.S.A.

Plasmin converts Scu-PA to two-chain urokinase by hydrolyzing the lys-158/ile-159 peptide bond. Using site directed mutagenesis, the codon at amino acid position 158 was changed from one that codes for lysine to one that codes for either alanine, glutamic acid, or methionine. These DNA constructions were expressed and amplified in Chinese hamster ovary cells. The resulting protein products were isolated and characterized in vitro. Under conditions where Scu-PA is completely converted by plasmin to two chain urokinase, none of these derivatives were cleaved by plasmin. However, all of these molecules, including Scu-PA, were cleaved by thrombin. These derivatives exhibited low amidolytic activity as determined by using the chromogenic substrate S2444 and low fibrinolytic activity as determined by using fibrin plates. All three derivatives activated either glu-plasminogen or lys-plasminogen to plasmin in vitro but at rates significantly slower than that seen with Scu-PA.