

THE THROMBOLYTIC ACTIVITIES IN A RABBIT MODEL OF VENOUS THROMBOSIS OF AN ACYL HUMAN PLASMIN DERIVATIVE (BRL 26920) AND AN ACYL STREPTOKINASE HUMAN PLASMINOGEN ACTIVATOR COMPLEX (BRL 26921) R.J. Dupe, P.D. English, J. Green and R.A.G. Smith Beecham Pharmaceuticals, Great Burgh, Epsom, Surrey, U.K.

The structures of the novel acyl-enzyme fibrinolytic agents BRL 26920 and BRL 26921 are given. A model of venous thrombosis in which thromboplastin-induced thrombi are formed in the inferior vena cava of the rabbit is described. Thrombi were held in place by an implanted woollen thread and thrombolysis was measured by release of ^{125}I -FDP into the circulation and by radiochemical quantitation of residual clots. BRL 26920 was compared with the same dose of activator-free human plasmin in the model. The acyl form of the enzyme was found to be significantly more active and caused significantly less disturbance of haemostasis than the free enzyme. Release of radioactivity from labelled thrombi suggested that the duration of fibrinolysis following a bolus of BRL 26920 was at least 4 hours. Similar comparisons of BRL 26921 with unmodified streptokinase human plasminogen activator complexes were made in this model. Although neither agent caused large haemostatic changes the acyl-enzyme was significantly more active.

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PHYSICAL, CHEMICAL AND IMMUNOLOGICAL PROPERTIES OF PORCINE AND HUMAN PLASMINOGEN ACTIVATORS OF VARIOUS TISSUES AND CELLS. E. R. Cole and R. M. Snopko. Section of Hematology, Rush-Presbyterian-St. Luke's Medical Center, Chicago, IL 60612, U.S.A.

Properties of plasminogen activator (PA) from heart, kidney, lung and plasma euglobulin of the pig and pig kidney cell culture were compared with the properties of PA from human heart, lung, urinary urokinase (UK) and human kidney cell culture (Abbokinase, AK). The isoelectric points of PA's were in the pI range of 7.0-7.8, except for UK and AK which had higher pI, 8.8-9.2 for the major forms. Mol. Wt. estimation by gel filtration revealed all PA, except AK, to have Mol. Wt. 48,000-54,000 with additional low Mol. Wt. forms of 25,500 and 36,000 for pig kidney cell culture PA and UK respectively. A single 35,500 form was seen for AK. Antiserum prepared against highly purified pig heart activator quenched the PA activity of pig heart, kidney, lung and euglobulin, but was inactive against pig kidney cell culture PA and UK. While pig heart PA is a poor activator of purified plasminogen and hydrolyzes AGLME poorly, pig kidney cell culture PA, UK and AK rapidly activate plasminogen and have high AGLME hydrolysis activity. A cofactor for pig heart PA (E.R. Cole, 1977, Blood 50: 262) which markedly accelerates plasminogen activation by pig heart PA, does not increase plasminogen activation by pig kidney cell culture PA, UK, AK or pig euglobulin and is instead slightly inhibitory. This data suggest that PA extracted from tissues is primarily in an inactive form, has little plasminogen activation and AGLME hydrolysis activity and is sensitive to cofactor due to unfolding of the PA with exposure of the active site. The urinary and cell culture forms of PA, in contrast, are released from cells in the active form, are insensitive to cofactor and have high plasminogen activation and AGLME hydrolysis activities.

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ULTRASONIC VIBRATION FOR BOOSTING FIBRINOLYTIC EFFECT OF UROKINASE. Shunro TACHIBANA. Division of Stroke Study, Wakasugi Medical Research Institute and Hospital, Sasaguri, Fukuoka, Japan

Boosting effect of ultrasonic vibration on fibrinolytic activity of urokinase has been investigated. Fibrinolysis was studied grouply in (1) experimental fibrin clot with urokinase, (2) clot with urokinase preceded exposure of clot to ultrasonic waves, (3) clot exposed to ultrasonic waves and (4) clot with saline solution. Scanning electro microscopic examinations were made on the clots in each four series. Fibrinolysis was not evident in the clots (3) and (4), though fine cracks were seen in the clots (3). The four hour rate of fibrinolysis was 50% in the clots (1) where as 100% in clots (2).

Procedures (1)(2)(3) and (4) were also employed in the Chandler's Loop Technique clot of four hour rotation. The rate of fibrinolysis was 50% in the clot (1) when 60 IU of the urokinase was used, where as 86% in the clot (2), which scanning electro microscopically gave a feature of decreased fiber structure. Fibrinolysis was accelerated by ultrasonic vibration in the clot with certain amount of urokinase added, breaking up of the clot being reached in shorter time than the series without ultrasonic wave application.

Ultrasonic vibration mechanically might cause micro-stream in the clot architecture, which could help urokinase contact directly with the fiber net. Scanning electro microscopic examinations revealed morphological representation of enhanced fibrinolysis by ultrasonic vibration. It was suggested that physical ultrasonic vibration could be applied to boost a chemical fibrinolytic effect of urokinase.

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DEXTRAN SULFATE STIMULATED FIBRINOLYTIC ACTIVITY IN WHOLE HUMAN PLASMA: DEPENDENCE ON THE CONTACT ACTIVATION SYSTEM AND ON A UROKINASE-RELATED ANTIGEN. Lindsey A. Miles, Zuleika Rothschild, and John H. Griffin Research Institute of Scripps Clinic, La Jolla, CA 92037 USA and University of São Paulo, Ribeirão Preto, Brazil

The generation of fibrinolytic activity in whole human plasma in the presence of dextran sulfate was studied. Plasma was preincubated with N-flufenamyl- β -alanine to remove its antiplasmin and antiactivator activities and then incubated with 50 $\mu\text{g}/\text{ml}$ dextran sulfate ($M_r \sim 500,000$) for 30 min at 40. The initial fibrinolytic activity in 30 min, as assessed on a ^{125}I -fibrin plate, was equivalent to approximately 9 ng/ml purified plasmin. A fraction of goat antibodies to plasminogen blocked the fibrinolytic activity of stimulated plasma, indicating that the activity was plasminogen dependent. Plasmas genetically deficient in either prekallikrein or Factor XII (Hageman Factor) showed a diminished initial rate of generation of fibrinolytic activity in response to dextran sulfate. However, after prolonged incubation (~3 hr) of stimulated deficient plasmas with fibrin, the fibrinolytic activity approached that of stimulated normal plasma. When normal plasma was preincubated with the gamma fraction of goat antibodies made against purified urokinase, the dextran sulfate stimulated fibrinolytic activity was markedly decreased in a dose dependent manner. The data suggest that the fibrinolytic activity stimulated in whole human plasma in the presence of dextran sulfate and N-flufenamyl- β -alanine is dependent upon proteins of the contact activation system and also upon molecules immunologically related to urokinase.