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LEECH PROSTAGLANDINS AND THE ENZYME DESTABILASE AS THROMBOLYTIC AGENTS OF THE PREPARATIONS FROM THE MEDICINAL LEECHES. I. Baskova, G. Nikonov, Laboratory of Blood Coagulation, Department of Biology, Moscow State University, Moscow, USSR

The thrombolytic effect of the leeching of thromboflebits has been well known from the ancient time. It is provided by the properties of the salivary gland secretion though leech saliva does not show proteolytic activity and does not activate plasminogen to plasmin. But in leech saliva we have found the enzyme destabilase (isopeptidase-glutaminase) which hydrolyze the cross-linked fibrin. This mechanism can be the basis of a new type of fibrinolysis. We have observed the high affinity of destabilase to fibrin that provides the dissolution of thrombus in circulating citrate blood in experiments *in vitro*. The high affinity of destabilase to fibrin correlates with its high ability to bound L-lysine, which inhibits isopeptidase and glutaminase activity of destabilase. Using radioimmunoassay for 6-keto-PgF<sub>1</sub> we have found prostaglandins in saliva and other preparations from the medicinal leeches:

3400 pg/ml of leech saliva  
322 pg/mg of protein (the whole leeches extract)  
351 pg/mg of protein (the head region extract)  
63 pg/mg of protein (blood from the intestinal tract)

But the leech prostaglandins in contrast to 6-keto-PgF<sub>1</sub> has strongly inhibited platelets aggregation stimulated by thrombin. The leech prostaglandins stimulate thrombolysis in rats after intravenous injection or oral administration. We have supposed that thrombolytic effect of leech prostaglandins is induced by the release of tissue plasminogen activator from vessel wall.

In the experiments on rats it has been shown that thrombolytic effect of leech saliva and preparations from the medicinal leeches is provided by the summary effect of the leech prostaglandins and the enzyme destabilase. After the extraction of prostaglandins by ethylacetate thrombolytic effect is diminished by 40 per cent.

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PROPERTIES OF THE COMPLEX BETWEEN  $\alpha_2$ -MACROGLOBULIN AND THE PROTEINASE BRINASE FROM ASPERGILLUS ORYZAE. L.-J. Larsson (1), E.P. Frisch (2) and I. Björk (1). Dept. Veterinary Medical Chemistry, Swedish Univ. Agricultural Sciences, Uppsala (1) and Dept. Blood Coagulation Disorders, Karolinska Hospital, Stockholm, Sweden (2).

Brinase, a proteinase from *Aspergillus oryzae*, has previously been shown to have a significant thrombolytic effect in patients suffering from peripheral arterial disease. Brinase is rapidly bound to  $\alpha_2$ -proteinase inhibitor and  $\alpha_2$ -macroglobulin ( $\alpha_2$ M) in plasma. Since binding of a proteinase to  $\alpha_2$ M only results in sterical blocking of the active site and not complete inactivation of the enzyme, it has been suggested that brinase in complex with  $\alpha_2$ M may retain ability to digest fibrin. To test this hypothesis, we have characterized the binding of brinase to  $\alpha_2$ M and the properties of the complex formed. Analyses by several techniques showed that one molecule of  $\alpha_2$ M can maximally bind two molecules of brinase. The bait region and the thioester bonds of  $\alpha_2$ M were cleaved in this reaction in a similar manner as in the reaction with trypsin. Moreover, a conformational change highly similar to that caused by trypsin was induced in  $\alpha_2$ M by brinase, as shown by changes of fluorescence emission, far-u.v. circular dichroism and u.v. absorption difference spectra. Thus, brinase appears to bind to  $\alpha_2$ M in the same manner as other proteinases. The activities of free brinase and two forms of brinase- $\alpha_2$ M complex, produced by reaction of the two components in an equimolar ratio or by saturation of inhibitor with enzyme, were compared by two different assays with high-molecular-weight substrates, i.e. hide powder azure or fibrin. The complex formed with equimolar amounts of brinase and  $\alpha_2$ M showed ~15% of the activity of free brinase in both assays, whereas the complex formed at saturation of inhibitor with enzyme showed ~35% of the free brinase activity. Although the brinase- $\alpha_2$ M complex, like other  $\alpha_2$ M proteinase complexes, is eliminated rapidly from plasma, the ability of the complex to cleave high-molecular-weight substrates may be an explanation of the thrombolytic effect of brinase seen in patients.

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THROMBOLYTIC EFFECTS OF A POLYANIONIC COMPOUND (PENTOSAN POLYSULPHATE SP54) IN GERIATRIC PATIENTS. Vogel, G. and M. Machulik. Department of Haemostaseology Medical Academy Erfurt, G.D.R.

In geriatric patients the use of streptokinase or urokinase is often limited by contraindications, particularly hypertension. As it has been demonstrated by different authors, the polyanionic compound pentosan polysulphate SP 54 induced an activation of both intrinsic and extrinsic pathway of fibrinolysis and produced a mild decrease of blood pressure. From these characteristics pentosan polysulphate SP 54 should be an ideal thrombolytic agent in geriatric patients. To clarify this, an open prospective study was performed. 24 patients (16 females, 8 males) age 65 - 78 years, suffering from thromboembolic processes (8 deep vein thrombosis (DVT), 16 thrombosis of retina vessels (TRV) were included. Pentosan polysulphate SP 54 (BENE-PHARMA München, F.R.G.) was administered by intravenous infusion 300 mg daily over a period of 10 days. Venography or ophthalmoskopie were repeated at day 11.

Results:

	complete lysis	incomplete l.	no lysis
DVT	4	1	3
TRV	8	5	3

Side effects: Bleeding 0 ; thrombocytopenia 2

It is concluded from these results, that pentosan polysulphate SP 54 is an useful thrombolytic agent in particular in geriatric patients suffering from hypertension.