

INTERMITTENT INFUSIONS OF ARVIN AND LOW-DOSE UROKINASE IN THE TREATMENT OF DEEP VEIN THROMBOSIS. M.K. Dubiel, M.F. Scully, R. Ham, B. Djazaeri and V.V. Kakkar. Thrombosis Res. Unit, King's College Hospital Med. School, London, England.

A preliminary study has been made on the thrombolytic therapy effectivity in seven patients with deep vein thrombosis, undergoing intermittent arvin/urokinase therapy. On the first day, patients received an infusion of 70 units of arvin over a period of 6 hours, followed by a bolus infusion of 70 units of arvin and half an hour later, a bolus infusion of 250,000 units of urokinase. After 6 hours 35 units of arvin were administered over a six hourly period followed by a bolus infusion of 250,000 units of urokinase. Subsequent four days consisted of a six hourly infusion of 35 units of arvin followed by a bolus infusion of 250,000 units of urokinase. Urokinase dose was repeated twice at 8 hourly intervals. Blood samples were collected each day, pre arvin, post arvin and post first dose of urokinase.

No haemorrhagic complications were observed. Two out of four patients with incomplete occlusive thrombi showed 100% lysis, in the other two no lysis occurred. The three patients with complete occlusive thrombi showed no lysis.

After the initial infusion of arvin, circulating clottable fibrinogen fell to 35% of the preinfusion level. Urokinase had a small effect on the fibrinogen level reducing it to 12% of the preinfusion level. Plasma concentration of fibrinogen degradation products initially rose to levels >3 mg/ml but dropped to lower levels remaining there throughout the treatment. On the first day of treatment plasminogen levels dropped by 60% and antipiasmin levels by 80% of the pretreatment level, after the administration of arvin and urokinase. Levels of urokinase rose to >0.3 u/ml post treatment but fell to <0.01 u/ml in the pretreatment samples.

These results suggest that this type of treatment involving a defibrinogenating agent arvin, and a plasminogen activator urokinase, may constitute a safe and effective way of lysing non-occlusive deep vein thrombin without any associated haemorrhagic complications.

RELEVANCE OF PLASMA ALPHA 2 ANTIPLASMIN MEASUREMENT FOR MONITORING THE OCCURRENCE OF A THROMBOLYTIC STATE DURING UROKINASE THERAPY. AN IN-VITRO STUDY. V. Musumeci, B. Zappacosta. Department of Internal Medicine, Università Cattolica del Sacro Cuore, Roma, Italy.

The aim of the present study was the search of changes in laboratory parameters which can be reliably correlated to the development of an effective thrombolytic state during urokinase therapy. Artificial radiolabelled venous thrombi were prepared by leaving native blood (0.1 ml) to clot in glass tubes for 2 hours in presence of $1-2 \mu\text{Ci}$ of ^{125}I fibrinogen (Sorin Saluggia, Italy). The labelled clots were introduced into a perfusion chamber (volume 0.2 ml) provided with a plastic retention screen and placed in the focus of the detector of a Pitman Ratemeter model 235 N connected to a recorder. The perfusion chamber was connected in a close circuit through a peristaltic pump to a reservoir containing citrated whole blood (15 ml) maintained at 37°C under continuous stirring. Blood was pumped through the chamber at a flow rate of 1 ml/min. Urokinase was introduced in increasing amounts in the reservoir during a period of 5 hours and at various intervals aliquots of blood (0.3 ml) were drawn and assayed after centrifugation for plasminogen, alpha 2 antipiasmin and fibrinogen. Alpha 2 antipiasmin was assayed by using Coatest Antipiasmin from Ortho Diagnostics. Plasminogen was assayed after SK activation by using the substrate S 2251 from Ortho Diagnostics. Results showed that an effective thrombolytic states, as detected by a decrease of clot radioactivity, appeared when alpha 2 antipiasmin concentration fell at levels below 40%. The alpha 2 antipiasmin measurement could be useful for monitoring the thrombolytic effectiveness of non-standard low or moderate dosages of urokinase therapy.

THE EFFECT OF ALPHA-TOCOPHEROL ON UROKINASE. D. Ogston. Department of Physiology, University of Aberdeen, Aberdeen, Scotland.

Alpha-tocopherol (vitamin E) in the form of its succinate markedly inhibited the activity of urokinase (5 CTA u/ml) on fibrin plates when tested at a concentration of 10^{-5}M . The amidolytic (S-2444) and esterase (N- α -acetylglucyl-L-lysine methyl ester) activities of urokinase were also inhibited by α -tocopherol. Inhibition was concentration and time-dependent. Sodium succinate (10^{-4}M) did not influence urokinase activity.

The inhibition of urokinase by α -tocopherol was abolished in the presence of dilutions of whole plasma. All fractions of plasma separated by gel filtration on Sephadex G-200 interfered with the inhibition of urokinase by α -tocopherol indicating that the effect of plasma does not reside in a single protein. The inhibition of urokinase by α -tocopherol could be reversed by serum albumin.

It is concluded that α -tocopherol is a potent inhibitor of urokinase in purified systems, but the effect of protein on the reaction suggests that this inhibition has little physiological relevance.

RAPID ANALYTICAL TECHNIQUES FOR MEASURING THE HIGH AND LOW MOLECULAR WEIGHT CONTENTS OF UROKINASE SAMPLES.

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Two assay procedures have been developed for measuring the ratio of the two molecular weight forms of urokinase commonly found in either urine or the fluid of cultured human kidney cells. The first method is a mini gel filtration technique which rapidly separates the high molecular weight (HMW) and low molecular weight (LMW) urokinases. This procedure can be used for urokinase samples of any purity. The biological active fractions are assayed by the fibrin plate technique. These biological activities are converted to relative molar amounts by the use of the molar activity constant for each molecular weight form. The second procedure is based on the differing responses of the two molecular species in the fibrin plate and the Ploug clot lysis assay systems. The comparison of assay responses of an unknown with a predetermined curve obtained from known mixtures of HMW and LMW urokinases results in a measure of the relative amounts of the two molecular forms. This system is not applicable to samples of low purity.

The two systems gave comparable results. For example, the WHO First International Reference Preparation (66/46) of Urokinase is 31% HMW urokinase by the differential method and 32% HMW urokinase by the gel filtration technique. Both methods require small amounts of sample (400 IU or less) and should replace the use of sodium dodecylsulfate polyacrylamide gel electrophoresis for urokinase molecular species determinations.