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CYCLIC THROMBUS FORMATION IN RABBIT AORTA: A NEW MODEL OF ACUTE ARTERIAL THROMBOSIS M. Kuhn, T.H. Müller, W.G. Eisert. Department of Biological Research, Dr. Karl Thomae GmbH, 7950 Biberach, Federal Republic of Germany

Existing models of arterial thrombosis either require extensive surgical intervention or are limited by the short period of observation. Therefore we developed a new model in rabbits to monitor cyclic flow reductions (CFR) due to thrombus formation in a partially denuded and stenosed aorta.

An electromagnetic flowprobe was positioned proximally to a stenosis of the abdominal aorta in anesthetized New Zealand rabbits after limited denudation of the vessel using a hemostat. The aortic blood flow was spontaneously reduced due to formation of thrombi. It returned to the basal level after gently shaking the occluder. The 'free flow' (defined as mean area under the flow curve) as well as the frequency of CFR were monitored. CFR persisted in all control animals for 3 to 4 hours. Prostacyclin at an infusion rate of 100 to 250 ng/kg/min completely abolished CFR in 16 of 20 animals. I.v. application of nitroglycerine (0.7 mg/kg/h) or papaverine (2 mg/kg/h) did not affect CFR. This as well as histology indicates strong involvement of platelet aggregation rather than vasospasm. CFR were also reduced by the following drugs: phosphodiesterase inhibitors (AHP 719, 1 mg/kg/h, i.v.; UDCG 212, 5 ug/kg/min., i.v.), a cyclooxigenase inhibitor (sulphinpyrazone, 30 mg/kg, i.v.) or a thromboxane synthetase inhibitor (dazoxiben, 1 mg/kg, i.v.) as well as an alpha2 antagonist (yohimbine, 1 mg/kg, i.v.). Heparin (800 U/kg, i.v.) reduced CFR suggesting significant contribution of the coagulation system.

This new approach opens an avenue to long term observations of antithrombotic therapy in a fairly simple and highly reproducible model.

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CATABOLIC BEHAVIOUR OF RECOMBINANT TISSUE PLASMINOGEN ACTIVATOR (R-t-PA) IN THE RAT. R. Harris (1), L. Garcia Frade (2), S. Poole (1), M. Mahmoud (1), A.D. Curtis (1) and P.J. Gaffney (1). National Institute for Biological Standards and Control, London, U.K. (1) and Ramon y Cajal Hospital, Madrid, Spain (2).

To compliment ongoing experimentation on the use of tissue plasminogen activator (t-PA) as a thrombolytic agent in a rat thrombosis model, clearance data for labelled R-t-PA in the rat were investigated. Both Iodogen and Chloramine T labelling procedures for ^{125}I incorporation yielded similar biological activity in the resultant labelled t-PA, while the half-lives in the rat circulation for Iodogen- and Chloramine T-labelled materials were 5½ and 7½ minutes respectively. Clearance data using promoter based and ELISA assays support that obtained with ^{125}I-labelled t-PA. Following fractionation of various timed rat plasma samples by HPLC on a gel exclusion column (TSK G-3000 SW), 90% of the labelled t-PA was distributed between two inhibitor/t-PA complex peaks in the 1 minute sample. One of these peaks (about 30% of labelled t-PA) was compatible with a t-PA/PAI-1 complex, having a molecular weight of about 120,000, while the other (comprising about 60% of labelled t-PA) had a molecular weight of about 350,000 and was undetectable by ELISA, bioimmunoassay or fibrin plate assay, while showing low activity by a promoter-type t-PA assay. The two major activity peaks in these HPLC profiles of rat plasma were associated with low levels of radiolabelled t-PA and were compatible with free t-PA and a complex of t-PA having a molecular weight of about 200,000. It was observed that free t-PA was retarded during the HPLC column separations and eluted as a broad trailing peak despite the presence of 0.1% - 0.5% Tween-80 in the column. Thus molecular weights of the various complexes formed are subject to further examination. All three t-PA peaks had the same initial half-life of 2-3 minutes. Since about 60% of the t-PA is contained in the high molecular weight inhibitor complex, we propose that the formation of this complex may be a major mechanism by which t-PA is cleared from the rat circulation, despite inactive catabolic breakdown products reappearing in the circulation following clearance of these complexes.

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A NEW PAN SPECIES MODEL FOR THE MEASUREMENT OF IN VIVO THROMBOLYSIS. A. Hotchkiss (1), C. Refino (1), L. DeGuzman, (1), B. Rigter (2) and W. Eisert (2). Genentech Inc, So. San Francisco, CA (1) and Dr Karl Thomae, GmbH, Biberach, West Germany (2).

A new model suitable for the quantitation of in vivo thrombolytic efficacy was developed which has significant advantages over currently available models. This model allows animals to be used repeatedly and produces data that are less variable.This is a species-independent model utilizing an arterio-venous shunt (AVS). This model was used in rabbits (3 kg) and beagle dogs (13 kg). Each animal was used repeatedly during a 7 day experimental period without evidence of antibody formation. Clot lysis data are less variable than the rabbit jugular vein clot lysis model (RJV). The average coefficient of variation at all doses ([SD/Mean]x100) with the AVS model was 13+9 and 21+6 for dogs and rabbits, respectively, compared to 25+11 for the RJV model. The rate of data accumulation with the AVS is 3-fold greater than the RJV.

In the dog, catheters (25 cm long, 1.6 mm ID) were placed in the jugular vein and the carotid artery, tunneled under the skin to the back of the neck, exteriorized and plugged with a heparin lock. Thrombi were made from a donor dog by placing 0.7 ml of whole blood in a 1 cc plastic syringe barrel containing a cotton thread. The blood was clotted for 2 hr at 37.5 deg C, in vitro. The thrombus was rinsed and the syringe barrel was connected into the circulation by silicone tubing for a 90 min test period. Clot lysis was determined by weight. The flow rate across the thrombus was regulated to 60 ml/min by placing a teflon capillary tube (30 cm, o.7 mm OD) on the venous side. The procedure for the rabbit study differed as follows: Rabbit blood made larger clots therefore the blood was diluted with saline (4 to 1). Clots were incubated 90 min in vitro. The flow rate was 40 ml/min. Clot lysis was measured by the loss of 125-I fibrinogen.

The dose of recominant t-PA (Activase^{R}, Actilyse^{TM}) that achieved 40% lysis (ED40) was determined to be 180 ug/kg in the dog and 660 ug/kg in the rabbit AVS model. Using the RJV model the ED40 was 340 ug/kg.

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PLASMINOGEN ACTIVATOR ACTIVITY (PAA) IN THE ISOLATED PERFUSED RAT KIDNEY (IPRK). W. Müllbacher, M. Maier and B.R. Binder, Lab. Clin.Exp. Physiol., Dept. Med. Physiol., Univ. Vienna, Austria

Isolated rat kidneys perfused with albumin elaborate PAA into the urine as well as into the perfusate as assessed by the fibrin plate technique. Urinay PAA can be inhibited by addition of a monospecific antibody directed towards urokinase (uPA) while the activity in the perfusate is inhibited by an antibody against tissue type plasminogen activator (tPA). Infusion of plasminogen (45ug/g/min) into the albumin containing perfusion fluid is followed by the appearance of plasmin in the urine and perfusate and doubles PAA in the urine. Kidneys perfused with both plasminogen and the anti-urokinase antibody (0.5mg) excrete similar PAA and plasmin into the perfusate while these activities in the urine are completely abolished. Replacement of the anti-urokinase antibody by an anti-tPA antibody (0.5mg) abolishes PAA as well as plasmin activity in the perfusate and PAA in the urine is significantly decreased. From these data it is concluded that PAA in the urine of the isolated perfused rat kidney is due to urokinase and PAA in the perfusate is caused by tPA. The results suggest that plasmin activity in the perfusate might trigger increased secretion of urokinase into the urine.

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