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IN VITRO STUDIES ON THE BINDING OF TISSUE-TYPE PLASMINOGEN ACTIVATOR (t-PA) AND UROKINASE (u-PA) TO LIVER MEMBRANES. N.K. Kalyan, S.G. Lee, W-T. Hum, R. Hartzell, M. Levner, and P.P. Hung. Microbiology Division, Wyeth Laboratories Inc., P.O. Box 8299, Philadelphia, PA 19101, USA.

The plasminogen activators, t-PA and u-PA, are glycoproteins known to be involved in homeostasis of the blood clotting system, and thus are of potential clinical use in the treatment of thrombosis. Several in vivo studies have shown that both t-PA and u-PA are quickly removed from the blood circulation, predominantly by the liver. The mechanism by which the liver removes these proteins is not understood. To delineate this, we conducted in vitro studies of binding of PAs or their derivatives to isolated mouse liver membranes utilizing a functional assay developed in our laboratory. The assay consisted of initial binding of t-PA to liver membranes followed by centrifugation to pellet the membranes and the assay of the activity of the membrane-bound t-PA by a fibrin-agar plate method. The bound t-PA, which retained complete enzymic activity, could be dissociated by SDS treatment in an undegraded form as shown by SDS-PAGE. The binding of t-PA as well as u-PA was very fast and did not compete with glycoproteins or sugars containing the terminal galactose, mannose and N-acetylglucosamine residues. Furthermore, the treatment of t-PA with neuraminidase and/or periodate oxidation did not affect its binding characteristics. These data suggest that the carbohydrate moieties of t-PA and u-PA, unlike many glycoproteins, do not mediate their binding to the liver. This raised the possibility of the liver binding sequence being located in the protein backbone, especially the non-protease domains which are known to determine the biological specificities of PAs. The relative binding of u-PA and its low molecular weight (LMW) derivative containing only the protease domain, to the liver membranes was studied. Unlike u-PA and t-PA, LMW-urokinase did not bind significantly. This suggests that the protein sequence containing the non-protease domains, rather than the carbohydrate moieties of PAs contain the information necessary for binding to the liver and possibly their clearance from the blood circulation.

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IMMUNOASSAYS FOR SINGLE CHAIN URINARY-TYPE PLASMINOGEN ACTIVATOR (SCUPA) IN PLASMA AND IN CELL CULTURE SUPERNATANTS. M. Mahmoud (1), F. Hammerschmidt (2), H. Scheuerlein (2), and P.J. Gaffney (1). National Institute for Biological Standards and Control, London, U.K. (1) and Sandoz Research Institute, Vienna, Austria (2).

Monoclonal antibodies (mabs) to SCUPA have been generated in balb/C mice by conventional means and have been demonstrated to have no crossreactivity with two chain urinary-type plasminogen activator (TCUPA). These mabs have been used to develop two types of specific assay for SCUPA. Mabs coated on polyvinyl plates in conjunction with polyclonal antibodies (pabs) to TCUPA have allowed the development of a catcher-tag ELISA using alkaline phosphatase-labelled goat anti-rabbit IgG as a final step. The sensitivity range of the assay was 0.5 - 10.0 iu/ml. A second bioimmunoassay (BIA) using SCUPA mabs as catcher and in-plate development with glu-plasminogen and S-2251 has yielded an assay with a sensitivity range of 0.5 - 10.0 iu/ml. The international unitage ascribed in these assays was derived by comparing the hydrolysis of S-2444 by the I.S. for TCUPA with the purified SCUPA following full activation with plasmin.

Using these assays it was found that normal pooled plasmas contained about 1.0 iu of SCUPA antigen which was fully inhibited such that no activity was evident by the BIA assay for SCUPA. This suggests that urokinase in plasma is present in two forms: SCUPA bound to inhibitor and TCUPA which is biologically active when assayed using a BIA based on immobilised pabs to TCUPA. Cell supernatants from cultured human lung fibroblasts yield a SCUPA/TCUPA ratio of 70/30 using S-2444 chromogenic assay following a plasmin-mediated SCUPA-TCUPA conversion step. It was also shown that, in these cell supernatants, SCUPA was secreted with no inhibitor bound to it, since the BIA and ELISA data were quite similar. A curious feature of these assays, which is as yet unexplained, is the observation that urokinase (both plasmin activated SCUPA and TCUPA), when immunologically adsorbed on to the PVC-immobilised specific mabs or pabs used in this study, readily activated plasminogen but showed no hydrolytic activity on the chromogenic substrate, S-2444.

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CONTENTS OF PHYLLOQUINONE AND MENAQUINONE FAMILY IN SERUM AND FECES FROM HUMAN NEWBORN INFANTS. A. SHIRAHATA (1), A. ASAKURA(1), T. NAKAMURA (1) and K. YAMADA (2). Dept. of Pediatrics, School of Medicine, Univ. of Occupational and Environmental Health, Japan (1) and Dept. of Pediatrics, School of Medicine, St. Marianna Univ., Japan (2).

A sensitive and specific method for the determination of phylloquinone (PK) and menaquinone families (MK-n), between MK-4 and MK-10, was developed. Vitamin K (VK) was extracted from serum and feces with n-hexane-ether, and purified with silica gel column and short alumina column. Eluted VK was separated by high performance liquid chromatography using Cosmosil 5 C_{18} column with ethanol-water as a mobile phase. The separated VK was detected by a fluorometry after its reaction with ethanolic sodium borohydride in a reaction coil connected by one-line to a chromatographic column. Minimum detectable quantities of PK, MK-4 and MK-7 were 0.1, 0.1 and 0.15 ng/ml, respectively.

In normal adult serum, PK, MK-4, MK-5, MK-6, MK-7 and MK-8 were detected. The mean±SD values of PK and MK-n from normal adult serum were 3.0±1.4 (PK), 0.3±0.2 (MK-4), 0.9±0.4 (MK-5), 0.3 ±0.3 (MK-6), 3.8±1.4 (MK-7) and 0.3±0.2 (MK-8)ng/ml, respectively. On the other hand, VK was not detected in 19 umbilical blood samples, excluding 3 samples in which 0.7, 1.0 and 2.5 ng/ml of PK were detected. In 7 healthy newborn infants under 8 days of age, VK was not detected excluding 3 cases. Of these 3 cases, in one case, 3.16 ng/ml of PK was detected, and in the other two cases, 1.1 and 1.5 ng/ml of MK-7 were detected. In 11 infants at one month of age, PK (10 cases) and MK-7 (5 cases) were detected, and mean±SD values of PK and MK-7 in the detected group were 0.55±0.44 and 0.62±0.18 ng/ml, respectively. VK was not detected in plasma of infants with primary hemorrhagic disease of the newborn (4 cases) and infantile vitamin K deficiency (2 cases). With a wide variation of VK content in each samlpe, PK and all MK families were detected in feces from normal adults. On the other hand, only PK and MK-7 were detected in meconium collected from 6 normal newborn infants before initial feeding. The contents of VK in meconium were less than one percent of those in adult feces. These results indicate that VK supply from maternal side in utero and from intestinal flora after birth is very poor in newborn infants.

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THE POSTNATAL DEVELOPMENT OF THE COAGULATION SYSTEM IN THE PREMATURE INFANT. M. Andrew (1), B.A. Paes (1), R.A. Milner (2), P.J. Powers (3),M.Johnston,(1) and V. Castle (1). Depts. Pediatrics (1), Epidemiology (2), Medicine (3), McMaster University, St. Joseph's Hospital, Hamilton, Ontario, Canada.

A cohort study was performed to determine the postnatal development of the coagulation system in the "healthy" premature infant. Mothers were approached for consent and a total of 132 premature infants were entered into the study. The group consisted of 64 infants with gestational ages of 34-36 weeks (prem 1) and 68 infants whose gestational age was 33 weeks or less (prem 2). Demographic information and a 2 ml blood sample were obtained on days 1, 5, 30, 90, and 180. Plasma was fractionated and stored at -70°C for batch assaying of the following tests: screening tests, PT, APTT; factor assays (biologic (B)); fibrinogen, II, V, VII, VIII:C, IX, X, XI, XII, prekallikrein, high molecular weight kininogen, XIII (immunologic (I)); inhibitors (I), antithrombin III, α_2-antiplasmin, α_2-macroglobulin, α_1-antitrypsin, C1 esterase inhibitor, protein C, protein S, and the fibrinolytic system (B); plasminogen. We have previously reported an identical study for 118 full term infants. The large number of premature and full term infants studied at varying time points allowed us to determine the following: 1) coagulation tests vary with the gestational age and postnatal age of the infant; 2) each factor has a unique postnatal pattern of maturation; 3) near adult values are achieved by 6 months of age; 4) premature infants have a more rapid postnatal development of the coagulation system compared to the full term infant; and 5) the range of reference values for two age groups of premature infants has been established for each of the assays. These reference values will provide a basis for future investigation of specific hemorrhagic and thrombotic problems in the newborn infant.

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