Measurements in Fibrinolysis

Level 5 – Green Side (Hungerford Foyer)

Free Poster Session 11.30 – 12.45

P5-031 0104 EVALUATION OF AN AUTOMATED AMIDOLYTIC ANTIPLASMIN ASSAY

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The manual antiplasmin (AP) assay (Teger-Nilsson, Scand. J. Clin. Lab. Invest. 1977) has been adapted for automatically performance on an Automated Kinetic Enzyme and Substrate analyzer. Comparison of the automated method with the manual method revealed good correlation (r=0.98). No significant differences were found when EDTA or citrate plasmas were used. Normal values from 160 healthy individuals were 101% (79%–125%). Addition of purified AT III to plasma had no effect on the AP assay. Also no effect was observed after infusion of AT III concentrates in patients with decreased AT III levels. In patients with liver cirrhosis AP levels were 74% ±14%, which were significantly higher than their AT III levels (62% ±12%). The same was observed in newborns, where the mean AP activity was 81% ±17%, while their AT III activities were 49% ±15%. From the AP assay in 985 patients only 5% (52) showed a decreased level (below 80%) and only 3% below 70%. For the AT III activities these figures were 20% and 12% respectively. Assay of both AP and AT III activity discriminates between Dissimilated Intravascular Coagulation and systemic fibrinolysis.

P5-032 0105 FLUOROGENIC PEPTIDE SUBSTRATES FOR PROTEASES IN BLOOD COAGULATION, KALLIKREIN-KININ AND FIBRINOLYSIS SYSTEMS: SUBSTRATE FOR PLASMIN AND FACTOR Xa

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Fluorogenic peptide substrates, peptidyl-4-methylcoumarin amides (MCA), are now commercially available and proved to be useful for the assay of α-thrombin, Factor Xa, urokinase, Limulus clotting enzyme, plasma and urinary kallikreins. We have newly synthesized the fluorogenic substrates for plasmin and Factor Xla and tested for their possible use as specific substrates for these enzymes. Among six peptidyl-Lys-MCA, Boc-Glu-Lys-MCA and Boc-Val-Leu-Lys-MCA were good substrates for bovine and human plasmins, which were activated by urokinase. Streptokinase-activated plasmin complex (molar ratio with 1 to 1) hydrolyzed the former but not the latter. Using Boc-Phe-Ser-Arg-MCA and Boc-Leu-Thr-Arg-MCA, a specific assay method for Factor Xla activated with Factor XIa in the presence of HMW kininogen and kaolin, was developed. The kinetic parameters of various proteinases towards the specific substrates were as follows: α-Thrombin (Boc-Val-Pro-Arg-MCA, Km=2.1x10⁻³M), Factor Xa (Boc-Ile-Glu-Gly-Arg-MCA, 1.6x10⁻⁷M), Plasma kallikrein (Z-Phe-Arg-MCA, 2.4x10⁻⁷M), Pancreatic kallikrein (Pro-Phe-Arg-MCA, 1.6x10⁻⁷M), Urinary kallikrein (Pro-Phe-Arg-MCA, 2.2x10⁻⁷M), Urokinase (Glutaryl-Gly-Arg-MCA, 4.4x10⁻⁷M), Limulus clotting enzyme (Boc-Leu-Gly-Arg-MCA, 2.7x10⁻⁷M), Plasmin (Boc-Glu-Lys-Lys-MCA, 6.7x10⁻⁷M and Boc-Val-Leu-Lys-MCA, 2.5x10⁻⁷M).