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EVALUATION OF COMMERCIAL PROTEIN C ASSAYS. W.M. Morrien-Salomons, A. Sturk, M.V. Huisman, J. Borm, H.R. BÜller, J.W. ten Cate. Division of Hemostasis and Thrombosis, Academic Medical Center, Amsterdam, The Netherlands.

Plasma protein C inactivates the activated coagulation factors V and VIII. The assay of protein C is important, because a protein C deficiency is associated with a thrombotic tendency. We therefore evaluated 5 commercial assays in 49 normal volunteers, 48 patients suspected of deep vein thrombosis (DVT) of the leg but with negative impedance plethysmography (IPG), and 52 patients with DVT proven by IPG. The assays were rocket electrophoresis (Merz and Dade antibody), ELISA (Boehringer Mannheim), 2 chromogenic activity assays (Behringwerke and Kabi) and a clotting assay (Behringwerke). Coumarin therapy was used by 13 DVT positive, and 3 DVT negative patients. Results are presented in the table.

	ELISA	ROCKET	K.CHROM	B.CLOT	B.CHROM
intra-assay VC	4.9	12.3	3.2	3.8	1.9
inter-assay VC	5.4	17.1	5.8	4.0	3.5
mean (% normal)	87	87	92	101	100
normal range	65-110	60-120	65-115	50-170	70-125
correlation 1	0.58	--	0.64	0.37	0.74
correlation 2	--	0.58	0.79	0.41	0.80

TABLE: In correlation 1 and 2, the assay results of all non-coumarin treated individuals (n = 133) were compared with rocket electrophoresis and ELISA resp.

In the non-coumarin treated patients, both in the DVT positive and in the DVT negative patient group one protein C deficiency was detected by all assays.

Based upon the large assay VC (ROCKET) and normal range (B. CLOT), and poor correlation of the assays with the ELISA (ROCKET, B. CLOT) we conclude that the ELISA, B.CHROM and K.CHROM are to be preferred. However, as B.CHROM does not need a plasma absorption step it is somewhat preferable for activity assays.

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STUDY ON THE EFFECT OF UNFRACTIONATED (UFH) AND LOW MOLECULAR WEIGHT (LMWH) HEPARINS ON THE DETERMINATION OF PROTEIN C ACTIVITY. N.Sala and J.Fontcuberta, Servei d'Hematologia, Hospital de Sant Pau, Barcelona, Spain.

In an attempt to see whether the presence of different heparins affected the determination of protein C activity (APC),this parameter was measured before and during treatment in 23 deep vein thrombosis patients that had been randomly treated with 3 different LMWH (Choay CY-216 and CY-222 and Kabi-2165) and UFH,for 10 days. Very low levels of APC (amidolytic assay that uses thrombin-thrombomodulin to activate the barium citrate eluted PC) were found in those patients receiving UFH and having an APTT more than 3 times that of control, as well as in those patients receiving LMWH CY-216 and having an APTT of only 8 to 10 seconds higher than that of control plasma. In patients receiving CY-222 and Kabi-2165, no significant differences were observed between APC levels before and during treatment. PC antigen (ELISA assay) was normal in all cases. In order to see if these low APC levels were due to interference of heparin with the assay and at which doses, control plasma pool was supplemented "in vitro" with 0 to 2,5 IU/ml (0 to 0,0025%) of UFH and with 0 to 8 anti-Xa U/ml of LMWH CY-216. APTT, PCAg, APC and presence of ATIII in the barium citrate eluates (immunodiffusion), were determined in all plasma samples before and after treatment with protamine sulphate (PS) at 0,003%. The results showed that UFH, when not neutralized with PS, resulted in low APC values only at doses higher than 0,8 IU/ml, corresponding to an APTT of more than 3 times that of control plasma. LMWH CY-216 at doses above 1 anti-XaU/ml, corresponding to an APTT of only 10 seconds higher than that of control,also produced a gradual decrease in APC values. ATIII was clearly visualized in the barium citrate eluates of all those plasma samples having a low APC value. The addition of PS to all samples containing UFH resulted in a complete normalization of APC values, with almost normal APTT values and disappearance of ATIII from the barium citrate eluates. On the contrary, addition of PS to plasma containing CY-216 resulted in low APC values and presence of ATIII in the eluates of those samples containing more than 4 antiXaU/ml, whose APTT still was about 10 seconds above that of control.

It is concluded that at therapeutic doses not only UFH but also LMWH CY-216 interfere with the APC assay, probably through binding of heparin-ATIII complexes to barium citrate and neutralization of the thrombin used to activate the barium citrate eluted PC. LMWH CY-222 and Kabi-2165, although increasing the APTT similarly to CY-216, do not seem to interfere with the APC assay. Protamine sulphate, at 0,003% in plasma, completely abolishes the effect of UFH on APC assay but not that of LMWH CY-216. More studies are being performed to see if higher doses of PS can be used to neutralize the effect of this LMWH.

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AMIDOLYTIC DETERMINATION OF ANTI-ACTIVATED PROTEIN C ACTIVITY IN PLASMA. F. Nicham, J.L. Martinoli. Serbio Lab., 92 Asnières, France.

Anti-activated protein C (anti-APC) potency of plasma was studied using purified bovine activated protein C (Bovine APC) and the chromogenic peptide substrate CBS 65.25. The choice of bovine instead of human APC was justified by a better sensitivity (Km = 0.14 and 0.42 mM respectively). Inhibition was shown to be dramatically enhanced by the presence of Heparin and calcium. No significant difference occurred for pH values up to 8.2 for both inhibition and hydrolysis reactions.

In the final test, 0.1 ml of 1:5 diluted plasma (Tris buffer saline, pH 8.4, containing 5 U/ml of Heparin) were incubated at 37°C with 0.2 ml of Bovine APC (0.125 U/ml). After 10 minutes of inhibition, 0.2 ml of CBS 65.25 (1.5 mM/l) were added to the mixture and the change in absorbance was recorded at 405 nm for 2 minutes. In these conditions linearity of the dose-response curve was ensured from 0 up to 130 % of activity (normal plasma pool being assigned to 100 %) ; day to day precision was 1.9 %. When a normal plasma was overloaded with different purified inhibitors such as antithrombin III, c1-esterase inactivator, alpha 2 macroglobulin, the measured anti-APC activities were not affected at all. It could be concluded that this test measures protein C inhibitor described by Suzuki.

Levels in 23 normal individuals averaged 97.7 %, giving a normal range of 77 - 118 %. Levels were below normal in 6 of 10 patients after surgery (54.1 +/- 4.8 %), in 18 of 19 patients with liver disease (49.5 +/- 9.6 %) and in 4 of 18 coumarin treated patients (54.9 +/- 6.5 %). In 9 of 10 patients previously characterized as type I protein C deficient, a statistically significant increase in anti-APC activity was observed (mean 110.7 +/- 7.7 %).

The use of a chromogenic peptide substrate has led to a sensitive and fast assay for anti-APC activity in plasma. That could be of interest in clinical investigations and knowledge of regulatory mechanisms in thrombotic disorders.