## 1503

ACTIVATION OF PROTEIN C INDUCES CHANGES IN ITS INTRINSIC FLUORESCENCE. A. Mosca (1), S. Viganò D'Angelo (2) and A. D'Angelo (2). Dipartimento Scienze Tecnologiche Biomediche, Università Studi Milano, Italy (1) & Coagulation Service, Istituto Scientifico S. Raffaele, Milano, Italy (2).

Upon activation with either thrombin (T) or thrombin-thrombomodulin complex (T-TM), the zymogen protein C (PC) is transformed into a serine-protease, activated protein C (APC), by release of a small activation peptide. The rate of PC activation changes dramatically with T or with T-TM as a function of the $Ca^{++}$ concentration in the activation medium, suggesting a configurational change of the zymogen in the presence of $Ca^{++}$. It has been shown that $Ca^{++}$ binding to one single high-affinity binding site of gla-domainless PC is accompanied by a significant decrease of the intrinsic fluorescence emission intensity of the protein and that the high-affinity binding site is retained following activation of gla-domainless PC (J. Biol. Chem: 258; 5554, 1983). In the present work we have investigated the fluorescence properties of PC in order to answer the following questions: 1) is there a difference in the fluorescence properties of PC as compared to APC? 2) is there a difference between the conformational changes of PC activated with T or T-TM? From our experimental data we conclude that : a) the fluorescence emission intensity of fully activated PC is about 54% of the PC zymogen fluorescence intensity ($\lambda$exc 280 nm, $\lambda$ em 345 nm, 0.6 $\mu$M PC or APC in 20 mM Tris-HCl, 0.1 M NaCl, pH 7.8 at 25°C); b) during activation of PC (2 $\mu$M) with T-TM (150 nM) in the presence of 2 mM $Ca^{++}$, there is a good correlation (r=0.959) between fluorescence quenching and degree of PC activation, as measured by the rate of cleavage of the chromogenic substrate S-2238; c) the maximal fluorescence quench of PC activated with T or T-TM are virtually identical. Preliminary data suggest that $Ca^{++}$ affects differently the fluorescence emission properties of PC and APC. These results suggest that evaluation of the fluorescence properties of PC might represent a valuable tool for the characterization of abnormal PC molecules.

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SUDAY ON A SUBSTANCE FUNCTIONALLY LIKE PROTEIN C IN PLASMA. I. TSUNEIZUMI, T. MEGURO, K. YAMADA, Department of Pediatrics, School of Medicne, St. Marianna University, Kanagawa, Japan.

The present report first deals with the isolation and characterization of a substance found to demonstrate protein C like activity(PCLA). When it was activated by thrombin,the PCLA substance prolonged the activated partial thromboplastin time(aPTT). The activated PCLA substance also hydrolyzed synthetic substrates such as S-2238,S-2366 and S-2266,while the PCLA substance was not cross reacted with anti-protein C serum(Behring Manheim). The PCLA substance was adsorbed by both aluminium hydroxide gel and barium sulfate. The level of Km,optimum pH and optimum I.S. in the amidolytic reaction with synthetic substrate is shown in the table.

| | Synthetic substrate | | |
|---|---|---|---|
| | S-2238 | S-2366 | S-2266 |
| Level of Km | $3.3\times10^{-5}$ | $2.7\times10^{-4}$ | $2.6\times10^{-4}$ |
| Optimum pH | 8.0-8.5 | 8.0-8.5 | 7.5-8.0 |
| Optimum I.S. | 0.1 | 0.1 | 0.1 |

In the chromatography of Sepharose CL-6B gel,the PCLA substance was eluted in the fraction of higher molecular weight,while protein C was eluted with a lower fraction.This prompts an estimate that the molecular weight of the PCLA substance is approximately 200,000. Through DEAE-Sepharose CL-6B gel ion exchange chromatography,the PCLA substance was eluted by a lower ionic strength than was protein C.
The levels of a PCLA substance were low in the patients with a vitamin K deficiency and in newborn infants. It is expected that our findings regarding the PCLA substance will be as clinically significant as that which we know about protein C at present.

## 1505

CONGENITAL SEVERE PROTEIN C DEFICIENCY IN ADULTS. S. Kakkar, E. Melissari and V.V. Kakkar. Thrombosis Research Unit, King's College School of Medicine & Dentistry, Denmark Hill, London SE5 8RX, UK.

We (Melissari et al, 1985, T.R. 29 [1985] 641) were the first to identify the occurrence of severe protein C deficiency in an adult with thrombophilia and undetectable protein C levels. This report documents our clinical and laboratory resuts of this patient and his family, as well as another 8 patients, in two more, unrelated families. In these unique families with members suffering from severe protein C deficiency (≤6%), no one had experienced neonatal purpura fulminans. Symptoms started mainly in their early twenties, except in 2 patients who first had symptoms at the ages of 11 and 13. The expression of the protein C deficiency was mainly recurrent superficial and deep iliofemoral vein thrombosis and pulmonary embolism. The protein C deficiency was also expressed as generalised peritonitis due to massive messenteric vein thrombosis, cavernus sinus, renal vein thrombosis and priapism. In one of these families, five members died of intra-abdominal thrombosis before the age of 40. A compensated diffuse intra- vascular coagulation syndrome was observed during massive thromboembolic attacks as evidenced by high levels of D-Dimer (≥5000ng/ml). The treatment of choice was heparin or urokinase (with the exception of one patient), followed by heparin and fresh frozen plasma. Long term prophylaxis was LMW heparin or low dose warfarin plus stromba. The one patient who did not respond to the thrombolytic treatment with urokinase was found to have in his plasma a high titre of inhibitor against urokinase and prourokinase. This patient responded to streptokinase treatment. D-Dimer levels in these patients in non-crisis state were raised and proportional to the degree of the protein C deficiency.

| Subject | D-Dimer steady state values (ng/ml) | % of protein C antigen |
|---|---|---|
| Normal adult control | 30 | 100 |
| Family members | 470 | ≤6 |
| " " | 100 | 25-15 |
| " " | 30 | 40-30 |

## 1506

THE CLINICAL SPECTRUM OF PROTEIN C DEFICIENCY IN A LARGE NEW ENGLAND KINDRED. E. Bovill, K.A. Bauer, P. Callas, B. West, J. D. Dickerman. University of Vermont College of Medicine, Departments of Pathology and Pediatrics, Burlington, VT, U.S.A. and Beth Israel Hospital, Harvard Medical School, Boston, MA, U.S.A.

A family with a high incidence of venous thromboembolism over six generations has been investigated. Medical histories have been obtained on 136 of the 368 members of the kindred which allowed assignment of individuals into positive, equivocal, or negative categories with respect to their thrombotic manifestations. Protein C levels were determined by antigenic assay. Patients with protein C levels less than 66% of normal (mean, minus 2 SD) were classified as having protein C deficiency.

| | PROTEIN C LEVEL | |
|---|---|---|
| HISTORY | % DEFICIENT | % NORMAL |
| Positive (N = 16) | 62.5% | 37.5% |
| Equivocal (N = 14) | 28.6% | 71.4% |
| Negative (N = 106) | 15.1% | 84.9% |

$X^2(2df) = 18.6$ ($p = .0001$)

The mean age of onset of thrombotic manifestations in the 10 protein C deficient patients with a positive history was 33.8 ± 16.4 years while the mean age of the deficient group with a negative history was 22.0 ± 10.9 years. We conclude that there exists a striking association between thromboembolic disease and protein C deficiency within this large New England kindred.