

Monday, July 13, 1981

Poster Presentations

Protein C - I

11:00-12:30 h

Grand Ballroom Lobby Boards 231-235

0178

ADHERENT MONONUCLEAR CELLS AND ACTIVATED PROTEIN C (APC).
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Addition of bovine APC to dilute whole blood or euglobulin assays of fibrinolytic activity produces little effect on rates of clot lysis. However, addition of APC to undiluted non anticoagulated or to 3.2% citrated whole blood followed by 125I-fibrinogen and thrombin produces clots which release fibrin degradation products and lyse within 24 hours. Ordinarily clotted non-anticoagulated whole blood does not lyse.

Addition of increasing amounts of APC from 12-100ug/ml increases the rate of whole blood clot lysis at least 20 fold over a 3.2% citrate control. This response is dose dependent with no evidence of saturation. Addition of increasing amounts of APC (25-200ug/ml) to platelet poor plasma (PPP) increases the rate of clot lysis only two fold. This increase is also dose dependent but is saturated at 80-100ug/ml. Addition of APC (60ug/ml) to 3.2% citrated PPP plus adherent mononuclear cells (1,000-2,000/MM³) again increases the rate of clot lysis at least 20 fold as with the whole blood system. However, this increase in rate is delayed. Substitution of platelet rich plasma (PRP) for PPP eliminates the lag phase. Substitution of non-adherent cells (1,000 MM³), RBC (3 million/MM³) or polymorphonuclear cells produces no acceleration of PPP clot lysis above the PPP-APC control. When deoxyglucose (1.4 x 10⁻¹M) and antimycin (2.9 x 10⁻⁵M) are added to the PPP plus adherent cells before APC, the cell dependent acceleration of clot lysis is abolished. Removal of plasminogen activator (PA) and plasminogen (P) from PPP by lysine agarose adsorption also inhibits the effect of APC and adherent cells whereas reconstitution restores activity to 60%.

We conclude that adherent mononuclear cells are required for expression of optimal APC pro-fibrinolytic activity and that this may require both P and PA. This suggests another role for mononuclear cells (monocytes) found in inflammatory and thrombotic lesions.

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EFFECT OF ACTIVATED AND NON-ACTIVATED PROTEIN C ON MOUSE TUMOR METASTASES. T.B. Gasic, J.L. Catalfamo & G.J. Gasic, Department of Pathology, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19104, U.S.A.

Platelet aggregation (PA) and fibrin deposition appears to be involved in the establishment of blood-borne tumor cells as metastases. Since it has been reported that activated Protein C have fibrinolytic activity or can inhibit coagulation, we investigated whether activated Protein C may inhibit tumor spread. Preliminary studies in the mouse system indicated that (a) activated Protein C prolonged the kaolin cephalin clotting time of plasma treated *in vitro* (>10min.) or of plasma obtained from *in vivo* treated mice (55 vs 30 sec.), (b) activated Protein C can inhibit PA by plasma membrane vesicles shed by metastasizing tumor cells; this effect was much greater with rat gel filtered platelets than with heparinized mouse PRP. The inhibition effect was also present *in vivo*, since 56 ug of activated Protein C, injected ip 15 min. before iv injection of tumor vesicles (200 ug protein), reduced significantly thrombocytopenia and signs of respiratory sickness produced by the vesicles.

After these studies, activated Protein C, as well as non-activated Protein C, was given ip 15 min. and 3 hr after iv inoculation of 5x10⁴ MCA mouse sarcoma tumor cells. Three weeks later mice were killed and lung tumors sized and counted. The data shown in the Table (average and range) indicate that activated Protein C decreases metastasis, while Protein C increases both the size and the number of tumors.

MICE TREATED WITH	LUNG TUMORS/MOUSE	TUMOR SIZE(mm)
Buffer	18 (1-60)	1.7 (1-2)
Activated Protein C	11 (3-24)	1.7 (1-3)
Protein C	28 (7-52)	2.9 (2-5)

While the presumptive effect of activated Protein C on metastasis might be related to its anticoagulant and anti-platelet activities, we know nothing how Protein C enhances metastases in our experiments.

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INHIBITION OF ACTIVATED PROTEIN C IN COMBINED FACTOR V/VIII DEFICIENCY. J.C. Giddings and A.L. Bloom, Department of Haematology, Welsh National School of Medicine, University Hospital of Wales, Cardiff, U.K.

Human prothrombin complex concentrate was chromatographed on a dextran sulphate/sepharose column to provide fractions with the recognised properties of protein C. Fractions treated with thrombin-coupled sepharose yielded samples which prolonged the activated partial thromboplastin time of normal human plasma and which had amidolytic activity against the chromogenic substrate S2238. A recent report indicated that normal human plasma inhibited this amidolytic activity whereas plasma from patients with hereditary combined factor V/VIII deficiency failed to do so (Marlar, R.A. and Griffin, J.H., J. Clin. Invest. 66: 1186, 1980). In the present study 21 plasma samples from patients of 11 different families with combined factor V/VIII defect were compared with normal plasma for their ability to inhibit the amidolytic action of thrombin-treated protein C. Six patients with classical severe haemophilia A and two patients with severe, isolated factor V deficiency were also studied. Plasma was mixed with activated protein C together with heparin and antithrombin serum. Aliquots were removed at intervals and incubated with substrate S2238. Hydrolysis was detected in a spectrophotometer at 405 nm. In eight separate experiments normal plasma inhibited an average 63.5% (±15.6) of the amidolytic activity in 30 mins. incubation. Plasma from patients with haemophilia A or isolated factor V deficiency gave results which were not significantly different from normal (67.2% and 61% respectively). However plasma from patients with the combined defect inhibited an average of 24.5% (±13.6) of the amidolytic activity (p<0.01). Two of these plasma samples failed to inhibit any protein C activity. The results confirm that normal plasma contains an inhibitor of activated protein C and this appears to be deficient in patients with hereditary combined factor V/VIII defect.