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IMMUNOCHEMICAL IDENTIFICATION OF A THROMBOSPONDIN -LIKE ANTIGEN IN ARTERIAL THROMBOGENIC MICROFIBRILS. F. FAUVEL-LAFEVE, Y.J. LEGRAND. Groupe de Recherches sur la thrombose et l'athérogénèse. U 150 INSERM, UA 334 CNRS, Hopital St. Louis, 75010 PARIS, FRANCE.

The biochemical structure of arterial microfibrils (MFS) is unknown. Presently, the most probable hypothesis is that elastin associated MFS contain several antigenic determinants with MW varying between 31 and 200 KD.
From our previous studies we know that MFS extracted by 6 M GuCl contain a major glycoprotein with a 128 KD MW (GP128). GP 128 is essential for the reactivity of MFS towards blood platelets but due tc the high insolubility of the extracted material it was not possible to isolate and study this GP 128. We have used immunoblotting to determine if MFS contain determinants recognized by antibodies against connective tissue glycoproteins such as fibronectin, type VI collagen or anti-platelet thrombospondin (TSP). The results showed that MFS do not contain fibronectin or type VI collagen but that anti-TSP IgG reacted with GP 128. Furthermore, the Fab fragments from anti-TSP IgG inhibited platelet aggregation induced by MFS but not by collagen or ADP . In a second step, to raise antibodies against GP 128, we prepared blots from entire MFS, the nitrocellulose band corresponding to GP 128 was cut, dissolved in DMSO, and was injected to rabbits. Such obtained antibodies recognized only GP 128 in arterial MFS and also TSP in a platelet lysate confirming that GP 128 and TSP have a common antigenic structure. IgG from anti-GP 128 inhibit platelet aggregation induced by MFS but not by collagen or ADP. Previously reported observations showed that tissue TSP and endothelial cells derived GP 128 have a similar affinity for chromatography supports and have the same effect on platelet-MFS interactions. All these results led us to propose that TSP, GP 128, and MFS recognize a common determinant on platelet membrane. This assumption would be strenghened if GP 128 indeed is derived from tissue TSP.

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THROMBOSPONDIN INTERACTION WITH PLASMINOGEN. Patricia DePoli, Theresa Bacon-Baguley and Daniel A. Walz. Wayne State University, Department of Physiology, Detroit, MI, U.S.A.

Platelet thrombospondin (TSP) interacts with plasminogen in a specific and saturable manner. TSP can form a trimolecular complex with histidine-rich glycoprotein and plasminogen and the plasminogen within such complexes can reportedly be activated by tissue plasminogen activator. We have studied the interaction of TSP with plasminogen using Western blotting of plasminogen, reduced plasmin and the elastase-generated fragments of plasminogen and their binding of iodinated TSP. TSP was found to specifically bind to plasminogen and the heavy (non-enzyme) chain of plasmin in a calcium-independent manner. Binding could be blocked by preincubation of the immobilized plasminogen or plasmin with an excess of unlabeled TSP. Plasminogen domains (kringles) were generated by limited elastase proteolysis. TSP bound specifically to a single 51 kDa plasminogen fragment. The elastase-generated fragments were separated by lysine-Sepharose chromatography and their identities established by amino acid composition and amino-terminal sequence analysis. The 51 kDa plasminogen fragment bound to lysine-Sepharose and had an amino-terminal sequence corresponding to kringle 4 (K4) and a composition consistent with that of K4-K5-plasmin. TSP binding to this fragment was not blocked by the presence of an excess of the fragment K1-K2-K3, K4, nor miniplasminogen (K5-plasmin). Binding does not appear to be directly dependent upon the specific high-affinity lysine binding site of the 51 kDa fragment. Our data suggests that thrombospondin interacts with plasminogen at a single distinct site, and that this recognition site is at or near the K4-K5 contiguous region of plasminogen.

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HAEMOSTATIC CHANGES INDUCED BY TWO LOW DOSE TRIPHASIC ORAL CONTRACEPTIVES. S.J. Machin, I.J. Mackie, K. Walshe, M.D. Gillmer. Haematology Department, Middlesex Hospital, London, W1, UK.

The haemostatic system was investigated in 26 women taking cyclically administered triphasic combined oral contraceptives for the first time during their first six cycles. Fourteen women received Logynon (mean dose 32.4µg ethinyloestradiol, 92µg progestagen) and 12 received SHD 415G (Schering) which contains a mean dosage of 32.4µg ethinyloestradiol and 78µg gestodene, a recently developed progesterone. The Logynon group showed a significant increase ($p<0.005$) in fibrinogen (pre-mean 284.4 g/l; after 1 cycle 347.3 g/l, after 6 cycles 318.6 g/l), factor VII (65.8 u/l to 73.9 u/l to 83.2 u/l), factor XII (1.74 u/l to 2.41 u/l, to 2.25 u/l), plasminogen (100.9 u/l to 135.1 u/l to 126.3 u/l); decrease in ATIII (115.9 u/l to 103.1 u/l to 93.4 u/l) but no significant change in factor X (98.4 u/l to 108.9 u/l to 102.4) or protein C (0.85 u/l to 0.88 u/l to 0.94 u/l) activity. The SHD 415G group showed similar changes with an increase in fibrinogen (247.9 g/l to 330.8 g/l to 373.1 g/l), factor VII (63.1 u/l to 73.1 u/l to 90.3 u/l, factor X (98.3 u/l to 112.0 u/l to 124.4 u/l), factor XII (1.46 u/l, to 1.93 u/l, to 2.03 u/l), plasminogen (110.8 u/l to 125.4 u/l to 136.7 u/l); decrease in ATIII (113.1 u/l to 96.3 u/l to 89.7 u/l), but no change in protein C (0.84 u/l to 0.78 u/l to 0.85 u/l) activity. These changes were apparent after the first cycle of therapy and the differences were maintained over the six cycle period. There was no increase in protein C activity despite changes in the other vitamin K dependent proteins factors VII and X. Both low oestrogen dose triphasic pills caused similar prothrombotic changes which were not modified by the new progesterone, gestodene.

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MOLECULAR MARKERS OF HEMOSTATIC ACTIVATION: COMPARISON OF PLASMA AND URINARY LEVELS. H.W. Ashley, J. Fareed. Loyola University Medical Center, Maywood, IL 60153

We have compared plasma and urine levels for both normal control and aspirin treated groups (n=11) using standard methods for fibrinopeptide A (FPA), platelet factor 4 (PF-4), β-thromboglobulin (β-TG), Bβ 15-42 peptide (BβP), and tissue plasminogen activator (t-PA). Normal males and females of varying ages (22-50) made up the two groups. For aspirin treatment, 300 mgs/day of aspirin was given PO for 3 days prior to the 24-hour urine and blood collection. The following data was generated:

	Plasma (ng/ml)		Urine (µg/24 hrs)	
Test	Normal	Aspirin	Normal	Aspirin
FPA	3.8±1.6	2.6±1.3	2.5±1.1	1.9±0.6
PF-4	8.5±7.1	5.6±4.4	-	-
TG	37.5±10.7	25.7±12.7	-	-
BβP*	19.9±5.8	10.7±4.3	31.5±29.7	15.5±7.5
t-PA	30.5±14.9	17.5±13.2	392±588	156±213

* Plasma - picomoles/ml; Urine - nanomoles/24hrs.

These results indicate that aspirin treatment produces a marked decrease of both plasma and urinary levels of molecular markers of hemostatic activation, suggesting that aspirin produces its effect at multiple sites within the hemostatic network. Profiling of urinary markers of hemostatic activation may also prove useful in the evaluation of prothrombotic states and evaluation of antithrombotic drugs.