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FACTOR XIII IS NOT INVOLVED IN THE PLATELET ADHESION TO COLLAGEN. K. Kariya, Y. Sawada, K. Ueno, I. Kudo, M. Aihara and Y. Yoshida. First Department of Internal Medicine, Hirosaki University School of Medicine, Hirosaki 036, Japan.

We have reported that the adhesion of human formalin-fixed washed platelets (FWP) to collagen was enhanced by von Willebrand factor but inhibited by plasma fibronectin (Thromb. Res. 44, 1986). Recently, platelet factor XIII is reported to be a receptor for collagen (Saito, JBC, 261, 1986). To investigate the role of factor XIII in the interaction between platelets and collagen, effect of purified XIII or rabbit anti-XIIIa on the adhesion of FWP to collagen or on the in vitro bleeding time was studies. FWP adhesion was measured by either collagen-coated glass beads column or aggregometric method using bovine type I collagen (Ethicon Inc., Dr. Kronenthal). In vitro bleeding time was measured with Thrombostat-4000 (VDG-VONDERGOLTZ), in which citrated whole blood as a sample and ADP, $CaCl_2$ and rat type I collagen as the reagents were used. Platelet adhesion to the collagen immobilized column (1,300 ug collagen, flow rate 5 ml/min) was not changed by the addition of purified XIII (Fibrogammin, Hoechst); the adhesion were 42.7 ± 1.7% in the presence of 1% human serum albumin, 42,0 ± 0.3%, 43,0 ± 1.4% in the presence of 1 or 2 u/ml factor XIII. Furthermore, the adhesion of FWP which was added by 1:100 rabbit anti-XIIIa was 42.3 ± 1.4% and not different from that of control rabbit serum (46.1 ± 1.3%). Similar results were also obtained with different technique using aggregometer. No significant change on in vitro bleeding time was observed after the addition of 1:100 rabbit anti-XIIIa to citrated normal blood. When the binding of factor XIII to the collagen was investigated by batch method, 17%, 23% and 54% of factor XIIIa in normal plasma bound to 250, 500 and 1000 ug/ml collagen, respectively. These data suggest that factor XIII is not involved in the platelet adhesion to the type I fibrillar collagen, while factor XIIIa in normal plasma binds to the collagen.

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A SENSITIVE ENZYME-LINKED IMMUNOSORBENT ASSAY(ELISA) FOR SERUM LAMININ. T.Sugihara (1), J.Takamatsu (1), T.Kamiya (1), H.Saito (1), K.Kimata (2), and K.Kato (3). 1st Department of Internal Medicine,Nagoya University,School of Medicine,Nagoya,Japan (1), Department of Chemistry,Faculty of Science Nagoya University (2), and Department of Biochemistry,Institute for Developmental Research,Aichi Prefectural Colony,Kasugai,Aichi,Japan (3).

Laminin, a large glycoprotein, is a major and specific component of basement membrane. There were little or no circulating laminin in normal persons, although some recent reports have showed increased values in various diseases(ex. diabetes mellitus and liver disease) by a radioimmunoassay(RIA) using laminin fragment. The minimum detectable sensitivity of RIA was reported to be 20 ng/ml of serum sample. We describe here a more sensitive immunoassay system, and also the concentrations of laminin in sera from healthy subjects and patients. A sandwich ELISA method for measurement of laminin was established by use of purified antibodies to mouse laminin. The assay system consisted of polystylene balls with immobilized antibody $F(ab')_2$ fragments and the same antibody Fab' fragments labeled with β-D-galactosidase from E.Coli. The assay was highly sensitive and can detect as small as 0.5 ng/ml of serum laminin. Coefficients of variation in within-run and between-run precision studies for serum laminin were good. Serum laminin levels in healthy subjects of various ages ranged from 1.5 to 3.9 ng/ml(n=60). Fifty eight patient sera (collagen disease(n=18), hepatic disease(n=20), and renal disease (n=20)) were examined. Significant differences between the normal sera and 3 diseases sera were observed as shown below.

		Laminin(ng/ml)	
Normal	(n=60)	2.48±0.66	
Collagen disease	(n=18)	3.48±0.68	**
Hepatic disease	(n=20)	3.47±2.15	*
Renal disease	(n=20)	4.59±2.68	**

* P <0.05
** P <0.001

It is concluded that circulating laminin apparently exists in normal persons and there are higher laminin level in some diseases which appears to involve basement membrane-rich organ.

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THROMBOGENICITY OF THE VESSEL WALL AFTER SINGLE AND REPEATED INJURY. **B. Pasche, J. Swedenborg and A. Ljungqvist. Departments of Experimental Surgery and Pathology, Karolinska Hospital, Stockholm, Sweden**

Injury to the endothelial lining of the vessel wall gives rise to increased thrombogenicity but fibrin formation is only seen after repeated injury.

The purpose of the present study was to simultaneously study the appearance of thrombin enzymatic activity and morphological changes on the injured vascular surface after primary and repeated injury. Endothelial injury was caused by balloon catheter in rabbits. The animals were sacrificed and the aorta was excised, inverted and mounted on a plastic rod. Thrombin enzymatic activity was measured on the surface by exposing it to either a chromogenic substrate ($thrombin_S$) or fibrinogen ($thrombin_F$). In the latter case generation of fibrinopeptide (FPA) was measured. Standard light microscopic procedures were also performed.

After injury no endothelial cells were seen. Thickening of the internal elastic lamina and accumulation of granulocytes and platelets was also noted. $Thrombin_S$ and $thrombin_F$ was detected on the surface. One week after injury the vessel wall had healed but the endothelial cells were metachromatic and had numerable mitoses. No thrombin activity exceeding the control was seen. After repeated injury $thrombin_S$ and $thrombin_F$ were again demonstrated but the fraction constituting $thrombin_F$ was larger than after primary injury. Microscopy at this time showed severe changes including vacuolisation.

The capacity of the surface to inhibit thrombin in vitro was also studied and it was found that this capacity was lower after repeated injury particularly in the case of $thrombin_F$.

It is concluded that injury of the endothelium gives rise to appearance of thrombin enzymatic activity. After repeated injury proportionally more $thrombin_F$ is found, which may explain why fibrinogen is more easily demonstrated on the surface after repeated injury.

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STUDIES ON SMALL MOLECULAR WEIGHT ADHESION PROTEINS (SAPs) FROM CONNECTIVE TISSUES. S. Wasi (1), P. Alles (1), D. Gauthier (1), U. Bhargava (2), J. Farsi (2), J.E. Aubin (2) and J. Sodek (2). National Reference Laboratory, Canadian Red Cross B.S. (1) and MRC Group in Periodontal Physiology, University of Toronto (2), Toronto.

We have identified a family of low molecular weight proteins with cell attachment properties in a variety of soft and mineralised connective tissues (Wong et al., Biochem. J. 232, 119, 1985). For further characterisation of these proteins we extracted porcine bones with 4 M guanidine hydrochloride and purified the proteins on a series of gel filtration columns. The purifed SAPs comprise three bands with Mr ≈ 14 000 - 17 000. All three proteins bound to heparin-sepahrose in both the presence and absence of 4M urea, and when eluted with 2 M NaCl they retained their cell binding capacity. These proteins promoted the adhesion and spreading of a variety of cell types, including normal fibroblasts, osteoblasts, and epithelial cells, and tumour (osteosarcoma) cells. On Western blotting SAPs did not cross-react with antibodies against fibronectin, laminin or type I collagen; however, they were recognised by a monoclonal antibody to human vitronectin, a polyclonal antibody to bovine vitronectin and polyclonal antibody to human somatomedin B. Dose response experiments indicated that maximum attachment of human gingival fibroblasts occurred in the presence or absence of fetal bovine serum on wells precoated with 2.5 $\mu g/cm^2$ of SAPs. Attachment of cells to these proteins was partially inhibited by the synthetic pentapeptide Gly-Arg-Gly-Asp-Ser. Utilising the nitrocellulose cell binding assay of Hayman et al (J. Cell. Biol. 95, 20, 1982), the cell attachment to these proteins could be completely inhibited by heparin (100 units/mL) whereas up to 1000 units/mL of heparin had no inhibitory effect on cell attachment to fibronectin and vitronectin. The occurrence of these proteins in a variety of connective tissues and their recognition by different cell types may reflect their general biological role in adhesive mechanisms in both hard and soft connective tissues. Currently, we are investigating the relationship between SAPs and vitronectin, since it is possible that SAPs represent a tissue-processed form of vitronectin or may be novel attachment proteins with regions of homology with vitronectin.