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CRYSTAL STRUCTURE OF A CLEAVED VARIANT OF HUMAN PLASMINOGEN ACTIVATOR INHIBITOR-1. Kathleen Aertgeerts, Hendrik L. De Bondt, Camiel De Ranter and Paul J. Declerck* Laboratory for Analytical Chemistry and Medicinal Physicochemistry and *Laboratory for Pharmaceutical Biology and Phytopharmacology, Faculty of Pharmaceutical Sciences, K.U.Leuven, Belgium.

The serine proteinase inhibitor (serpin) plasminogen activator inhibitor-1 (PAI-1) can adopt three different conformations: active, latent and substrate, each with different functional properties. To characterize the structural requirements for the conformational flexibility of PAI-1 we have solved the X-ray structure of a cleaved (at P1-P1'), human, substrate PAI-1 mutant (Ala→Pro at position P12; Audenaert et al., J. Biol. Chem. 269, 19559, 1994) to a 2.7 Å resolution.
The three dimensional structure revealed three main features. Firstly, the N-terminal side (P16-P1) of the reactive site loop was completeley inserted into β-sheet A, yielding a β-strand s4A. Secondly, and in contrast to the X-ray structure of latent PAI-1 (Mottonen et al., Nature 355, 270, 1992), a new β-strand s1C was formed from P6' to P10' (Ile 352-Arg 356). Thirdly, the presence of a relatively important flexibility in two loops close to s1C ("loop1" connecting s4C with s3C and "loop2" connecting s3B with hG) could be deduced. Whereas in the current structure the smallest distance (of the backbone atoms) between these two loops is only 7Å, it is 12Å in latent PAI-1.
Based on the 3D-structure of the intact and cleaved serpin ovalbumin, it is commonly accepted that a substrate-like serpin does not have the ability of forming β-strand s4A. However, our data clearly showed that the substrate behaviour of this PAI-1 variant is associated with the generation of a β-strand s4A. The evidence for the easy movement of the loops close to s1C provides a possible explanation for the lability of active PAI-1: during the transition of the residues P6'-P11' from the β-strand s1C (presumably also present in active PAI-1) to the loop in the latent conformation, the gap between loop1 and loop2 is enlarged due to a sterical hindrance (Coulombic and van der Waals repulsion) between P6'-P11' and loop1.
In conclusion, the X-ray structure of the cleaved variant of human PAI-1 provides new data further illustrating the uniqueness of PAI-1 within the serpin superfamily.

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TYPE 1 PLASMINOGEN ACTIVATOR INHIBITOR GENE EXPRESSION IS INCREASED IN PROLIFERATING ENDOTHELIAL CELLS. Irene M. Lang*', Gertraud Lang', Wolfgang Farber', Kenneth Moser^, and Raymond R. Schleef*. 'University of Vienna, Austria; ^University of California, San Diego, California, U.S.A.; and *The Scripps Research Institute, La Jolla, California, U.S.A.

Although abnormal thrombus formation plays a key role in a variety of cardiovascular disorders, little information is available on the local mechanisms that stabilize and further fibrin deposition in various pathological conditions. One current marker for a prethrombotic state is the plasma level of type 1 plasminogen activator inhibitor (PAI-1), the major inhibitor of tissue-type plasminogen activator. To determine whether the abnormal production of this fibrinolytic inhibitor is involved in both acute and chronic vascular thrombus formation, we have analyzed specimens harvested from patients with acute pulmonary emboli and patients undergoing pulmonary thromboendarterectomy for chronic thromboembolic pulmonary hypertension (CTEPH). For comparison, pulmonary arteries from organ donors were harvested and processed in a similar fashion. *In situ* hybridization and immunohistochemical analysis of acute pulmonary emboli revealed intense PAI-1 immunoreactivity and mRNA signal confined to intimal endothelial cells (ECs) in the close vicinity of the central fresh fibrin-platelet thrombus. These ECs appeared to invade into the thrombus from the margins, thus forming new lumina within the clot. Moreover, high levels of PAI-1 antigen and mRNA were detected in ECs lining red, fibrin-rich thrombi as well as neovascular structures within organized yellowish-white thromboemboli from patients with CTEPH. To clarify whether high PAI-1 gene expression within ECs in pulmonary arteries of patients with acute and chronic pulmonary thromboemboli was specific, pulmonary thromboemboli derived from a canine model of acute pulmonary embolism were analyzed. Similar intense PAI-1 immunoreactivity was detected in ECs lining 7 and 30-day-old canine thromboemboli. Positive immunoreactivity of these ECs with proliferating nuclear cell antigen demonstrated that the ECs lining fibrin-platelet thrombi were proliferating cells that were actively involved in the early organization process of the clots. It is concluded that increased PAI-1 expression within organizing vascular thrombi may be related to the proliferative state of pulmonary arterial ECs.

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PROTEIN KINASE C INHIBITORS AND GEMFIBROZIL PREVENT THE ENHANCING EFFECT OF VERY LOW DENSITY LIPOPROTEINS ON THE BIOSYNTHESIS OF PLASMINOGEN ACTIVATOR INHIBITOR TYPE 1 BY HepG2 CELLS. Elena Tremoli, Cristina Banfi, Luigi Sironi, Mauro Porta, Damiano Baldassarre, Luciana Mussoni. Institute of Pharmacological Sciences, University of Milan, Italy.

Triglyceride-rich lipoproteins (VLDL) have been previously shown to enhance the biosynthesis of plasminogen activator inhibitor type 1 (PAI-1) by HepG2 cells. This study was undertaken to assess whether the effect of VLDL on PAI-1 antigen and mRNA induction could be by protein kinase C (PKC) signaling pathway. To this end confluent HepG2 cells were first incubated for 16 h with VLDL isolated from normal donors, at the 100 µg/ml concentration with of without inhibitors of PKC. At the end of incubation PAI-1 antigen released in the conditioned medium was determined by ELISA and PAI-1 mRNA expression was assessed by Northern analysis. Exposure of HepG2 cells to 100 µg/ml VLDL resulted in a twofold increase in PAI-1 antigen release and total PAI-1 mRNA expression. H7 (50 µM) and sphingosine (3-5 µM) almost completely prevented (> 80%) the effect of VLDL on PAI-1 antigen release and total PAI-1 mRNA accumulation. In addition down regulation of PKC, obtained by preincubation of HepG2 cells with PMA (100 nM) for 24h, prevented the effect of VLDL on PAI-1 biosynthesis. Established that the effect of VLDL on PAI-1 bisosynthesis was mediated by activation of PKC signaling pathway we evaluated whether fibric acid derivatives influenced PAI-1 biosynthesis in unstimulated HepG2 cells and in cells exposed to VLDL. In unstimulated HepG2 cells, Gemfibrozil (0.1-0.75 mM) significantly reduced PAI-1 antigen release (-85 % at the 0.75 mM concentration) and mRNA expression, whereas Bezafibrate at the highest concentration used (1 mM) reduced PAI-1 antigen release by 20%, with no effect on PAI-1 mRNA expression. In VLDL treated cells, only Gemfibrozil, at the 0.75 mM concentration, attenuated (-50%) the biosynthesis of PAI-1 as induced by VLDL (100 µg/ml). It is concluded that VLDL enhance PAI-1 biosynthesis through activation of PKC and that Gemfibrozil, but not Bezafibrate, attenuates PAI-1 induction in these cells.

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THE CLEAVAGE AND INACTIVATION OF PLASMINOGEN ACTIVATOR INHIBITOR TYPE 1 BY NEUTROPHIL ELASTASE: THE EVALUATION OF ITS PHYSIOLOGICAL RELEVANCE IN FIBRINOLYSIS. Tetsumei Urano, Kai Wu, Hayato Ihara, Yumiko Takada and Akikazu Takada. Dept. of Physiology, Hamamatsu University School of Medicine, Hamamatsu, Japan

The effect of the proteolytic cleavage of plasminogen activator inhibitor type 1 (PAI-1) by human neutrophil elastase (HNE) on fibrinolysis was investigated. HNE cleaved active recombinant prokaryotic PAI-1 (rpPAI-1) resulting in the formation of low molecular weight forms of rpPAI-1 as previously reported (Levin & Santell, J. Cell Biol. 1987). The latent form of rpPAI-1 was resistant to HNE. NH_2-terminal sequence analysis indicated that the cleavage site was Val355-Ser356 (P4-P3). The fact that the strained loop of the latent form of PAI-1 is buried inside the molecule most likely accounts for its resistance to HNE. After the cleavage by HNE, active rpPAI-1 lost its specific activity toward plasminogen activators. The cleavage was under both enzyme concentration and time dependent, and the almost complete inactivation of rpPAI-1 (2.9 µM) activity was obtained by a HNE (83 nM) treatment for 30 min. at 37°C. The effect of PAI-1 cleavage by HNE on t-PA induced clot lysis was studied in a purified system. Clot lysis time without rpPAI-1 was 20.0 ± 5.0 min., and was prolonged to 86.7 ± 2.9 min. by 68 nM of rpPAI-1. It was shortened when HNE (from 0.6 nM to 80 nM) was added and returned to the value obtained without rpPAI-1 when 80 nM of HNE present (20.0 ± 5.8 min.). In the absence of PAI-1, however, the elastase did not enhance clot lysis at all. The cleavage and inactivation of PAI-1 by HNE was shown to be a novel pathway to enhance fibrinolysis.