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Abstracts

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PS-1460 Evidence for the relevance of PAI-1 neutralization to enhance fibrinolysis by thrombin and calcium bound factor Xa in the presence of vitronectin or heparin

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Plasminogen activator inhibitor type 1 (PAI-1), which primarily targets plasminogen activators (PAs), inhibits thrombin and calcium bound factor Xa (B.B.A. in press) in the presence of either heparin or vitronectin (Vn). PAI-1 activity toward PAs, in reverse, is neutralized by these enzymes. To assess physiological relevance of such phenomena, we analyzed the effects of either heparin or heparin on tPA induced fibrin clot lysis in a purified system. Fibrin clot was made by the addition of human thrombin (0.1–1.0 IU/ml, 5–50 nM) to the mixture of single chain tPA (0.1 nM), Glu-plasminogen (0.5 μM), fibrinogen (2 μM), 5 mM CaCl2 and recombinant prokaryotic PAI-1 (4 nM), either in the presence or absence of Vn (20 nM) or unfractioned heparin (5 U/ml). In the absence of PAI-1, the clot lysis time was 0.41 hours and was not changed by thrombin concentration. It was prolonged by the addition of PAI-1 at different extent depending on the concentration of thrombin (Table 1; control). It was shortened by either heparin or Vn (Table 1; heparin or Vn), whereas the clot lysis time in the absence of PAI-1 was not shortened by these modifiers. Factor Xa (10 nM) shortened the clot lysis time at low thrombin concentration (0.1 or 0.5 U/ml) further in the presence of PAI-1 especially when either heparin or Vn exist (Table 2), although such shortening was not observed in the absence of CaCl2 (Data not shown). Fibrin autography revealed that factor Xa quenched the complex formation between single chain tPA and PAI-1, liberating excess amounts of free tPA. Results obtained in the present study suggest that these enzymes enhanced fibrinolysis by neutralizing PAI-1 activity and releasing excess amounts of free tPA. Such phenomena seem to be physiologically relevant and to play an important role in cross talk between coagulation and fibrinolysis.

Table 1

	Th 0.1 u/ml	Th 0.5 u/ml	Th 1.0 u/ml
Control	43.8 ± 4.9	25.7 ± 3.7	13.9 ± 0.8
Heparin	43.9 ± 11.0	12.1 ± 2.6	3.6 ± 0.2
Vn	17.0 ± 1.6	1.9 ± 0.7	0.9 ± 0.0(hours)

Table 2

	Th 0.1 u/ml	Th 0.5 u/ml	Th 1.0 u/ml
Control	20.6 ± 0.8	15.0 ± 1.6	12.4 ± 0.2
Heparin	15.8 ± 0.4	7.9 ± 1.0	3.6 ± 0.4
Vn	6.8 ± 2.6	1.3 ± 0.2	1.2 ± 0.1(hours)

PS-1461 Significance of PAI-1 and tPA · PAI-1 complex as the indicator of the severity of ARDS

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Background: Abnormalities of coagulation and fibrinolysis are closely related to organ dysfunction. We studied the usefulness of plasminogen activator inhibitor-1 (PAI-1) and tissue plasminogen activator · PAI-1 complex (tPA-PAI) as the indicators of the severity of acute respiratory distress syndrome (ARDS).

Methods: Six patients with ARDS were studied (male 5, female 1. age:44 ~ 85 y.o.). Three patients died. Total number of the days evaluated were 28 days. Analysed items were, 1) PAI-1, tPA-PAI, 2) Fibrinogen (F), fibrinogen and fibrin degradation products (FDP), plasminogen (PM), α 2-plasmin inhibitor · plasmin complex (PIC), D-dimer, thrombin antithrombin III complex (TAT) as parameters of coagulopathy, 3) DIC score (DIC criteria from the Ministry of Health and Welfare of Japan), 4) Multiple Organ Failure (MOF) score (calculated from MOF criteria of Japanese Association for Critical Care Medicine) and PaO_2/FiO_2 ratio (P/F), as parameters of organ dysfunction.

Results: There was positive correlation between the the DIC score and the MOF score (r^2 = 0.38, n = 28). There were also positive correlation between the DIC score and tPA-PAI (r^2 = 0.37), and negative correlation between the DIC score and PAI-1 (r^2 = 0.49). Negative correlation was found between tPA-PAI and PAI-1 (r^2 = 0.33). tPA-PAI was positively correlated to MOF score (r^2 = 0.43), and negatively correlated to P/F (r^2 = 0.43). Correlation between PAI-1 and the listed parameters of organ dysfunction was quite reversed as compared to tPA-PAI. No significant correlation were found between MOF score and all of the listed parameters of coagulopathy but tPA-PAI and PAI-1.

Interpretation: Our data showed close relationships between tPA-PAI and most of the above-listed important parameters. Reversed relationships were found between PAI-1 and the parameters. PAI-1 and tPA-PAI seemed to be one of the most sensitive indications of the severity of DIC and ARDS.

PS-1462 Identification of plasminogen-binding site with which histidine-rich glycoprotein functions as an antifibrinolytic factor

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Background: Histidine-rich glycoprotein (HRG) is known to have a high affinity for plasminogen. However, the site for plasminogen-binding on HRG or its physiological significance has not been established yet.

Methods and Results: Carboxypeptidase Y digestion of HRG resulted in a time-dependent loss of a plasminogen-binding ability of the digest, which was parallel with a release of the C-terminal Lys. A series of synthetic peptides with the C-terminal 4 to 20 amino-acid sequence of HRG were examined for their abilities to inhibit the plasminogen-binding of HRG. C20 peptide, Gly-Lys-Phe-Lys-Ser-Gly-Phe-Pro-Gln-Val-Ser-Met-Phe-Phe-Thr-His-Thr-Phe-Pro-Lys showed a strong inhibition in a concentration-dependent manner and a 100-fold molar excess of C20 inhibited about 50% of the plasminogen-binding of HRG. Furthermore, a peptide as small as C7, Phe-Thr-His-Thr-Phe-Pro-Lys, had essentially the same inhibitory effect as C20. On the other hand, C'19 peptide which lacks the C-terminal Lys of C20 completely lost its inhibitory effect. These results clearly show that the C-terminal Lys is essential for the plasminogen-binding of HRG. Next, we examined the effect of HRG on a fibrinolytic activity of plasmin using fibrin plates. HRG preincubated with an equal molar concentration of plasmin inhibited 47% of the fibrinolytic activity of plasmin, while the C-terminal Lys-truncated HRG did not have any inhibitory effect. C20 peptide also produced a concentration-dependent antifibrinolytic effect, and a 50-fold molar excess of C20 peptide inhibited about 50% of the fibrinolytic activity of plasmin without any effect on its amidolytic activity.

Interpretation: HRG functions as an antifibrinolytic factor by binding to the lysine-binding site of plasmin (ogen) through its C-terminaly Lys, which prevents a binding of plasmin (ogen) to fibrin. These results indicate that not only a catalytic activity but also a fibrin-binding property of plasmin (ogen) is essential for its fibrinolytic activity.

PS-1463 Additive effect of triglycerides and insulin as determinants of PAI-1 plasma levels in normo and hypertriglyceridemic subjects

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Impaired fibrinolytic function, secondary to elevated levels of PAI-1, is frequently observed in patients with CHD, obesity, diabetes, hypertriglyceridemia, hyperinsulinemia and insulin-resistance syndrome. A direct correlation between PAI-1, triglyceride (TG) and/or insulin levels was found in normal subjects and in patients at risk of atherothrombotic disease. It is still open the question whether triglycerides and/or insulin are, among others, the major physiological regulators of PAI-1 plasma levels. In this study we have investigated the relationship between fasting levels of insulin, lipids and PAI-1 antigen in normo and hyperlipidemic subjects (n = 163). 44 subjects were characterized by TG levels below 150 mg/dl, 60 by TG levels between 151–350 mg/dl and 59 by TG levels higher than 351 mg/dl. In the overall group, PAI-1 plasma levels correlated positively and significantly with insulin, TG, body mass index, total cholesterol and blood glucose. At the stepwise multiple regression analysis, insulin and log transformed TG were the only significant and independent predictors of PAI-1 antigen levels (r^2 = 0.21, $p < 0.0001$). To better investigate the role of triglycerides and insulin as determinants of PAI-1, the subjects were categorized in tertiles according to TG and insulin levels and PAI-1 compared within strata of these parameters. PAI-1 antigen levels gradually increased with rising levels of TG within all strata of insulin. In contrast, the increase of PAI-1 with rising of insulin levels was evident in the highest tertile of triglycerides only. In addition, subjects in the lowest tertiles of TG and insulin had lowest PAI-1 antigen levels, while subjects in the highest tertiles of TG and insulin had the highest levels of PAI-1. In conclusion, an additive effect of triglycerides and insulin as determinants of plasminogen activator inhibitor-1 levels is suggested.

PS-1464 PAI-1 levels are decreased by insulin treatment in NIDDM

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Background: Relationship between hyperinsulinemia and increased PAI-1 level is well-known and has been explained by influence of insulin on PAI-1 synthesis in the liver. This opinion is not fully accepted because: 1/.Postprandial hyperinsulinemia does not cause PAI-1 level elevation during circadian rhytm, 2/. Acute or short-time insulin administration is not accompanied by increased PAI-1 level.