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Polymorphonuclear leukocyte lysosomal proteases, cathepsins B and D affect the fibrinolytic system in human umbilical vein endothelial cells

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Abstract

To clarify the physiological role played by neutrophil lysosomal protease in cultured human umbilical vein endothelial cells (HUVEC), we studied the effects of cathepsins B and D released from activated polymorphonuclear leukocytes on the fibrinolytic system in HUVEC. Cathepsins B and D reduced the antigens of tissue-type plasminogen activator, and they increased both the antigens and the activity of plasminogen activator inhibitor-1. These results suggest that cathepsins B and D are involved in the thrombotic tendency, since they inhibited the fibrinolytic system in cultured HUVEC.

Keywords: Cathepsin B; Cathepsin D; Tissue-type plasminogen activator; Plasminogen activator inhibitor-1; Human umbilical vein endothelial cell

Tissue-type plasminogen activator (t-PA) is a highly specific protease that is synthesized by vascular endothelial cells and secreted into the bloodstream. This enzyme plays a key role in the fibrinolytic system, the system that constitutes the natural counterpart of the blood coagulation system and is responsible for the timely degradation of fibrin structures in blood clots and thrombi [1].

Plasminogen activator inhibitor-1 (PAI-1) is also synthesized by vascular endothelial cells and secreted into the bloodstream. This protein is the main physiological inhibitor of both t-PA and urokinase-type plasminogen (u-PA) in plasma [2].

T-PA and PAI-1 synthesized in vascular endothelial cells are important for the regulation of the blood coagulation system in the bloodstream.

The migration of polymorphonuclear leukocytes (PMN-L) into tissue is involved in the central event in the inflammatory response [3–5]. Inflammatory tissue injury is induced by the abnormal release of lysosomal enzymes such as cathepsins B, D, and G from activated PMN-L [6–10]. Vascular injury causes a thrombotic tendency and disseminated intravascular coagulation (DIC) [11]. The fibrinolytic system in the bloodstream is regulated by a balance between t-PA and PAI-1 secreted in vascular endothelial cells.

We studied the physiological role played by the PMN-L lysosomal proteases, cathepsins B and D, in the fibrinolytic system in cultured human umbilical vein endothelial cells (HUVEC).

Third-passage cultures of HUVEC were used; they were incubated in a 5% CO_2 atmosphere at 37°C. Determination of t-PA and PAI-1 antigens and their activity in HUVEC was performed with enzyme-linked immunosorbent assay (ELISA) kits (Biopool Co.) and activity assay kits (SPECTROLYE™/fibrin, Biopool Co.), respectively. Briefly, HUVEC, grown to confluence in collagen-coated 6-well plates (Corning Glass Works), were then incubated for 3, 6, 12 and 24 h in EGM-UV culture medium (Kurabo Co.) with cathepsin B or cathepsin D (Sigma Co.). After incubation, t-PA and PAI-1 antigens and their activity in the supernatants were measured with the t-PA and PAI-1 ELISA kits and the activity kits, respectively.

As shown in Fig. 1, t-PA and PAI-1 antigen levels were increased, incubation time-dependently, in the cultured HUVEC. PAI-1 antigen levels were 41.5-, 23.8-, and 10.6-fold the level of t-PA antigen at 3-, 6-, 12- and 24-h

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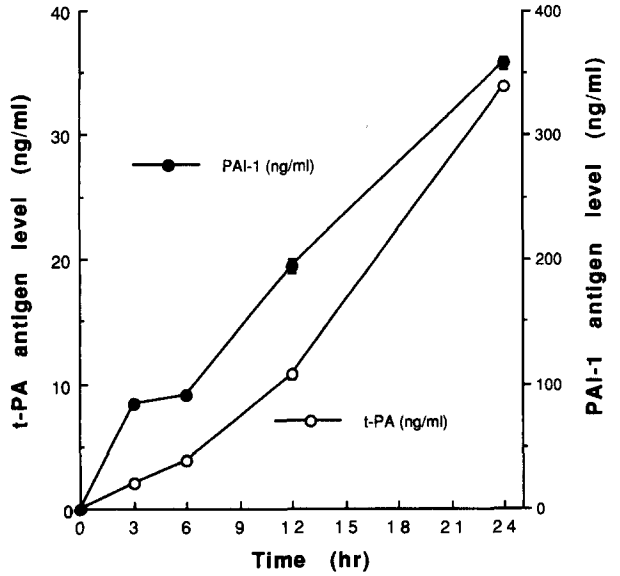

Fig. 1. Time-dependent release t-PA and PAI-1 in cultured HUVEC. Values are means ± S.E. of 6 experiments.

exposure, respectively. PAI-1 activity in conditioned medium-treated HUVEC (controls) also was 25.36 ± 1.59, 32.68 ± 8.83, 52.18 ± 7.03 and 49.20 ± 3.76 IU/ml (means ± S.E. of 6 experiments) at 3-, 6-, 12- and 24-h exposure, respectively. T-PA activity was not detected in this study. These results suggest that t-PA activity is inhibited by the binding of t-PA to HUVEC [12,13] and/or by the formation of t-PA-PAI-1 complex [13,14].

Fig. 2 shows the dose-dependency of the effects of cathepsin B on t-PA and PAI-1 antigen levels in cultured HUVEC. In 24-h incubation, cathepsin B (5.0 U/ml) significantly reduced t-PA antigen levels to 35% of control values (Fig. 2a). Conversely, in 24-h incubation, cathepsin B, at 1.0 U/ml and 5.0 U/ml, significantly increase PAI-1 antigen levels, 1.29-fold and 2.12-fold, respectively, compared with control values (Fig. 2b). In 12-h incubation, cathepsin B (5.0 U/ml) induced a 1.72-fold increase over control values (Fig. 2b).

As shown in Fig. 3a, the release of t-PA antigens was significantly ($P < 0.01$) reduced with 1.0 U/ml and 5.0 U/ml of cathepsin D at 12- and 24-h incubation. Fig. 3b shows the dose-dependency of the effects of cathepsin D on PAI-1 antigen levels in cultured HUVEC. Cathepsin D significantly ($P < 0.01$) and dose-dependently stimulated the release of PAI-1 antigens at 0.1 U/ml, 1.0 U/ml, and 5.0 U/ml with both 12- and 24-h incubation, respectively.

As shown in Fig. 4a, PAI-1 activity was increased 1.64- and 2.39-fold at 1.0 U/ml and 5.0 U/ml of cathepsin B, respectively, with 24-h incubation. Cathepsin D also increased PAI-1 activity, by 2.40-fold at 1.0 U/ml and by 2.75-fold at 5.0 U/ml with 12-h incubation, and by 2.73-fold at 1.0 U/ml and by 2.97-fold at 5.0 U/ml with 24-h incubation (Fig. 4b). The increase in PAI-1 activity induced by cathepsin D was greater than that induced by cathepsin B. Cathepsin B is a cysteine protease, and cathepsin D is an aspartic protease. Therefore, it seems likely that the differences in effects on t-PA and PAI-1 antigen release and PAI-1 activity exerted by cathepsin B and cathepsin D may be due to the differences in the types of proteases.

Gilboa et al. [15] reported that opsonized zymosan-activated neutrophils inhibited fibrinolysis. Recently, it has been reported that a PMN-L serine protease, cathepsin G, inhibits the fibrinolytic system by releasing PAI-1 from platelets and cultured HUVEC [16]. In acute inflammation, stimulation of leukocytes can lead to vascular injury. PMN-L are the most prominent cells at inflammatory sites during the early stages of inflammation and are thought to

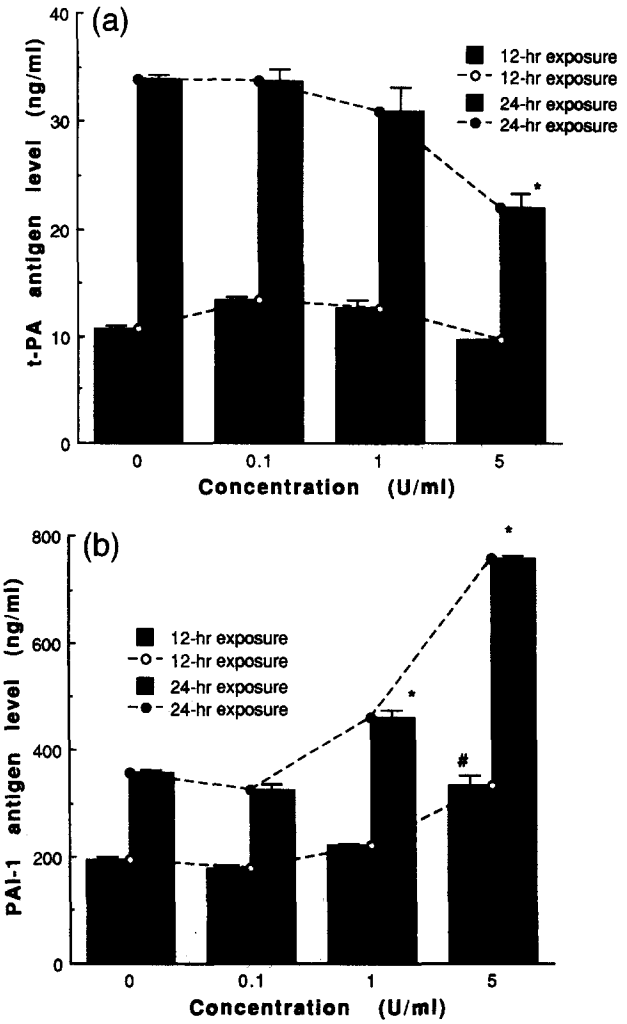

Fig. 2. (a) Effects of cathepsin B on t-PA release in cultured HUVEC. Values are means ± S.E. of 6 experiments. * $P < 0.01$, significantly different from control values at 24-h exposure. (b) Effects of cathepsin B on PAI-1 release in cultured HUVEC. Values are means ± S.E. of 6 experiments. # $P < 0.01$, significantly different from control values at 12-h exposure; * $P < 0.01$, significantly different from control values at 24-h exposure.

be responsible for injury. Vascular injury evoked by activated PMN-L occurs via at least two pathways. (1) Vascular injury is due to PMN-L adherence to endothelial cells, the formation of a protected microenviroment, the release of injurious inflammatory proteases such as cathepsins B, D and G, and elastase, and subsequent tissue injury [1]. (2) Vascular injury is induced by vascular obstruction brought about by PMN-L/PMN-L and PMN-L/endothelial cell adherence [2]. In this study, we found that the cysteine protease, cathepsin B, and the aspartic protease, cathepsin D, increased PAI-1 antigen levels and PAI-1 activity in cultured HUVEC, similarly to findings with the serine protease, cathepsin G [15]. These results suggest that cathepsins B and D released from activated PMN-L are involved in the thrombotic tendency, since these cathepsins inhibit the fibrinolytic system in cultured HUVEC by increasing PAI-1 antigen and PAI-1 activity, and by reducing t-PA antigen.

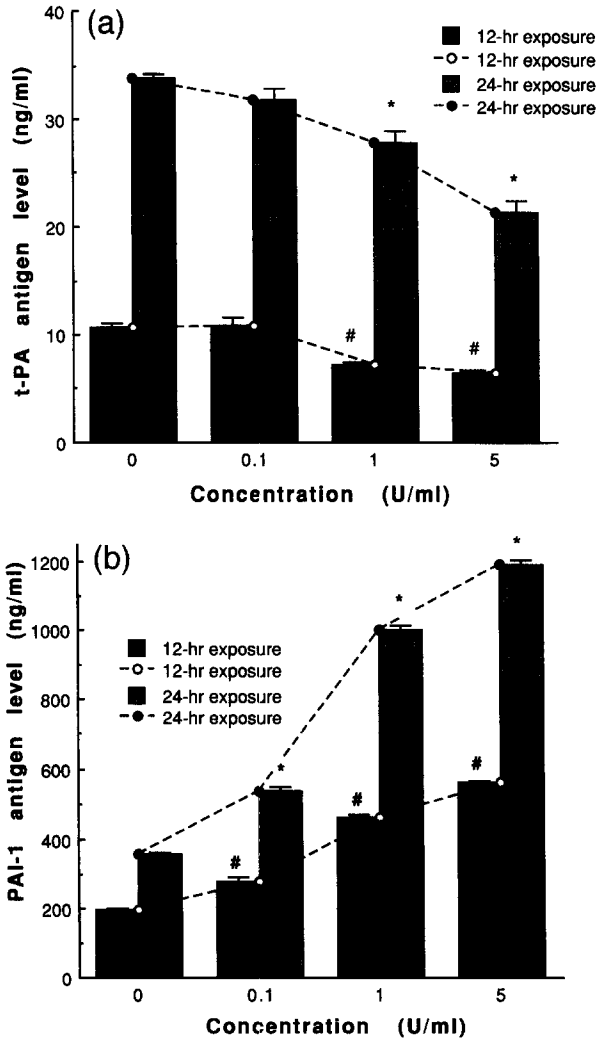

Fig. 3. (a) Effects of cathepsin D on t-PA release in cultured HUVEC. Values are means ± S.E. of 6 experiments. # $P < 0.01$, significantly different from control values at 12-h exposure; * $P < 0.01$, significantly different from control values at 24-h exposure. (b) Effects of cathepsin D on PAI-1 release in cultured HUVEC. Values are means ± S.E. of 6 experiments. # $P < 0.01$, significantly different from control values at 12-h exposure; * $P < 0.01$, significantly different from control values at 24-h exposure.

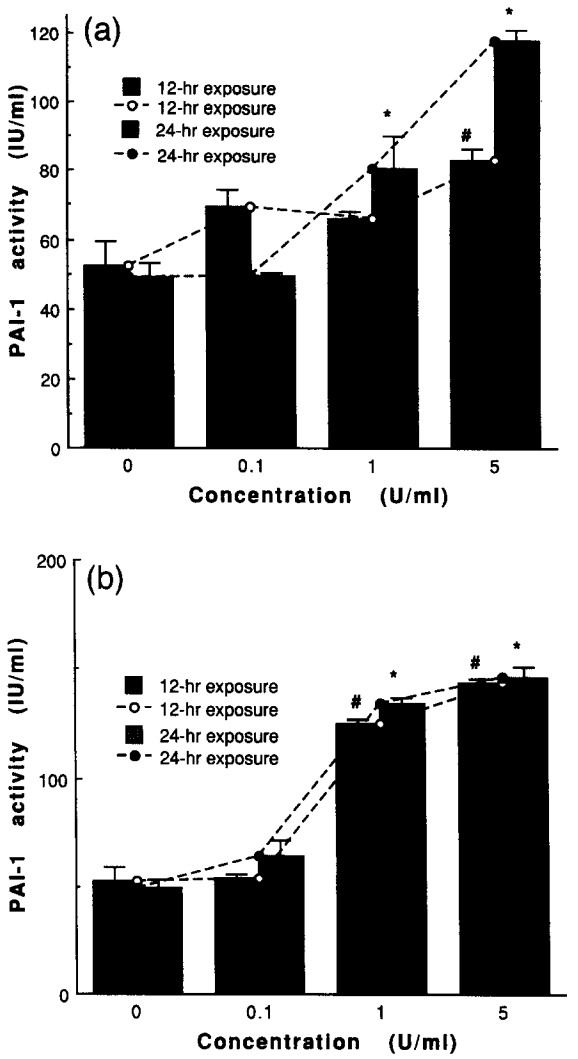

Fig. 4. (a) Effects of cathepsin B on PAI-1 activity in cultured HUVEC. Values are means ± S.E. of 6 experiments. # $P < 0.01$, significantlu different from control values at 12-h exposure; * $P < 0.01$, significantly different from control values at 24-hr exposure. (b) Effects of cathepsin D on PAI-1 activity in cultured HUVEC. Values are means ± S.E. of 6 experiments. # $P < 0.01$, significantly different from control values at 12-h exposure; * $P < 0.01$, significantly different from control values at 24-h exposure.

The mechanisms whereby cathepsins B and D evoke these effects on t-PA and PAI-1 production require further clarification.

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