TRANS-PROSTAGLANDIN E₂ ENHANCES FIBRINOLYSIS

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5,6-*trans*-Prostaglandin E₂ (*trans*-PG E₂) was found to accelerate the fibrinolysis in a cell-free system. *trans*-PG E₂ decreased the lysis time by 15% in the fibrin clot lysis time method and increased the lysis area by 22% in the fibrin plate method. On the contrary, 5,6-*cis*-prostaglandin E₂ did not have such effects. *trans*-PG E₂ did not exert the effect in the absence of either tissue-plasminogen activator or plasminogen. These results suggest that *trans*-PG E₂ enhances the generation of plasmin from plasminogen by tissue-plasminogen activator.

KEYWORDS — 5,6-*trans*-prostaglandin E₂; fibrinolysis; enhancement

INTRODUCTION

Prostaglandins (PGs) are widely distributed in biological fluids and tissues and exhibit a variety of activities. Most PGs isolated from natural sources have double bonds at C-5 and C-13, and, the Δ^5 PGs have dominantly *cis* configuration except for PG I₂ which have *trans* structure. The *trans*-PGs other than PG I₂ have been little noted on account of their relatively small biological activities compared with those of *cis* isomers. In the present study, we attempted the exploration of activities of *trans*-PGs using 5,6-*trans*-PG E₂ as a representative and found that 5,6-*trans*-PG E₂ significantly enhanced the fibrinolysis in a cell-free system.

MATERIALS AND METHODS

Chemicals — 5,6-*cis*-PG E₂ (*cis*-PG E₂) and 5,6-*trans*-PG E₂ (*trans*-PG E₂) were purchased from Funakoshi Chemical Industry Company, Ltd. (Tokyo, Japan). Human plasminogen (native type), fibrinogen and one chain form tissue plasminogen activator (tPA) were obtained from Kabi Vitrum Stockholm, Sweden. Thrombin was the product of Green Cross Co. (Osaka, Japan).

Fibrin Clot Method — The effect of *trans*-PG E₂ was examined by the fibrin clot method. The sample solution contained PG E₂ (either *cis* or *trans* form, 100 µg/ml), plasminogen (final concentration 100 µg/ml) and tPA (170 unit/0.31 µg/ml). Fibrin clot was prepared by mixing 50 µl of thrombin suspension (100 NIH U/ml), 250 µl of fibrinogen (4 mg/ml) and 100 µl of CaCl₂ (50 mM) in 10 mM sodium phosphate buffer pH 7.4 containing 0.14 M NaCl. After the clot formation was completed, an aliquot (100 µl) of the sample solution was added to the clot. The time required for the solubilization of the clot was recorded at 37 °C. The control from which PG was omitted was proceeded at the same time.

Fibrin Plate Method — The fibrin plate prepared with bovine fibrinogen (plasminogen removed) was purchased from Kitasato Institute Co. (Japan). Plasminogen (4 µg), tPA (14 unit) and either *cis*-PG E₂ (10 µg) or *trans*-PG E₂ (10 µg) were mixed and made up to 100 µl with 10 mM sodium phosphate buffer pH 7.4 containing 0.14 M NaCl. After the mixture was incubated at 37 °C for 3 min, an aliquot (10 µl) of the reaction mixture was applied to the fibrin plate. After incubation at 37 °C for 18 h, the degree of fibrinolytic activity was estimated by measuring the size of the lysis area.

RESULTS AND DISCUSSION

PGs E₁, E₂ and I₂ have so far been examined for the inhibition of platelet aggregation (1) or stimulation of tPA release from fibroblast (2). In the present study, we examined *trans*-PG E₂ for its effect on the fibrinolytic system in the absence of either platelet or cell. The effect of 5,6-stereoisomers of PG E₂ on the fibrinolytic activity of the plasminogen-tPA system was demonstrated by the fibrin clot lysis method consisting of purified fibrinogen and thrombin. Table I shows that *trans*-PG E₂ decreased the lysis time of fibrin clots to about 85% of the control value, whereas *cis*-PG E₂ decreased the lysis time by only 4% compared with control. When the *trans*-PG E₂ concentration was changed up to 100 µmol/ml of its final concentration, its decreasing effect on the lysis time reached a plateau at 20 µg/ml (data not shown). The effects of the isomeric PG E₂ were also tested using the fibrin plate method (Table I). Although *trans*-PG E₂ markedly increased the lysis area of fibrin plate, *cis*-PG E₂ did not influence the area. These enhancements of the fibrinolysis by *trans*-PG E₂ required the presence of both tPA and plasminogen (data not shown). *trans*-PG E₂, therefore, seems to increase the plasmin generation from plasminogen. Stereochemical property around C-5,6 olefinic linkage in PG E₂ may be the determining factor of this effect.

Table I. Effects of 5,6-Stereoisomer Prostaglandin on Fibrinolysis

	Lysis time (min)	Lysis area (mm ²)
Control	196.7 ± 1.5	77.65 ± 1.51
5,6- <i>cis</i> -PG E ₂	189.3 ± 1.5 *	81.45 ± 1.16
5,6- <i>trans</i> -PG E ₂	166.7 ± 7.8 *	94.82 ± 0.93 *

*: Significantly different from control (p<0.001).

Values are means ± SD of 3~4 samples.

The biological activity of *trans*-PG E₂ has little been noted so far. For example, the activities of the *trans* isomer in the inhibition of platelet aggregation and contraction of guinea pig ileum were less than those of the *cis* isomer or PG E₁ (3). On the other hand, Blair et al. (4) reported that various PGs increased the activity of adenylate cyclase in the homogenate of NCB hybrid cells. They compared the concentration for half-maximum activity (μM) of PGs activating adenylate cyclase. They showed that *trans*-PG E₂ (2.59 μM) was 12 times stronger than *cis*-PG E₂ (31.5 μM) and 1/100 time weaker than that of PG I₂ (0.0246 μM).

The present study revealed that *trans*-PG E₂ is a potent enhancer of the fibrinolytic activity compared with its *cis* isomer. The structure-activity relationship between C-5,6 olefinic linkage in PGs and its effect on fibrinolysis has been little studied. The present results suggest that modification of this region affects fibrinolysis. Gryglewski (5) examined the influence of PG I₂ and 6-keto-PG E₁ on fibrinolysis. Both compounds enhanced the fibrinolysis *in vivo* but *in vitro*, only 6-keto-PG E₁ shortened fibrin clot lysis time. These facts imply that the C-5,6 position of PGs is important for their effect on the fibrinolysis *in vitro*. The understanding of the effects of the C-5,6 modified PGs may be useful for unravelling the relationship between PGs and the fibrinolytic system.

REFERENCES

- 1) D. E. Vaughan, S. R. Plavin, A. Schafer and J. Loscalzo: PG E₁ accelerates thrombolysis by tissue plasminogen activator, *Blood*, 73, 1213-1217 (1990).
- 2) D. J. Crutchley, L. B. Conanan and J. R. Maynard: Stimulation of fibrinolytic activity in human skin fibroblasts by prostaglandins E₁, E₂ and I₂, *J. Pharmacol. Exp. Ther.*, 222, 544-549 (1982).

- 3) D. Van Dorp: Recent developments in the biosynthesis and the analyses of prostaglandins, *Ann. NY Acad. Sci.*, 180, 181-199 (1971).
- 4) I. A. Blair, C. N. Hensby and J. MacDermot: Prostacyclin-dependent activation of adenylate cyclase in a neuronal somatic cell hybrid: Prostanoid structure-activity relationships, *Br. J. Pharmacol.*, 69, 519-525 (1980).
- 5) R. Gryglewski: Prostacyclin-experimental and clinical approach., *Adv. prostaglandin, Thromboxane, Leukotriene Res.*, 11, 457-461 (1983).

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