The Influences of a Stable Analogue of TXA₂, 9, 11-epithio-11, 12-methano TXA₂ (STA₂), on Bovine Aortic Smooth Muscle Cells in Culture*

Michitaka NAITO**, Toshio HAYASHI**, Masafumi KUZUYA**,
Chiaki FUNAKI**, Kanichi ASAI** and Fumio KUZUYA**

TXA₂ is one of the main products of arachidonic acid cascade in platelets and has the strongest platelet aggregating and vasoconstrictive action among all substances which have ever had such an action. However, TXA₂ is extremely labile and its half life time is about 30 seconds in physiological conditions. So, there has been some difficulty in studying the role of TXA₂ on the initiation and development of atherosclerosis.

In this study, we report the effects of a stable analogue of TXA₂, STA₂, on cultured vascular smooth muscle cells (SMC).

Materials and Methods

We reported the method of cultivating SMC from fetal bovine aortas previously¹⁾. Cells subcultured 4 to 5 times were used for the experiments. Migration of SMC was examined by the method of Falk et al.²⁾, using a 48-well micro chemotaxis chamber (Neuroprobe). The supernatant of platelet lysate prepared by the method of Ohnishi et al.³⁾ was used as PDGF. PGI₂ released in the medium was assayed as its stable product 6-keto PGF_{1α} by radioimmunoassay as reported elsewhere⁴⁾.

Results

STA₂ itself had no chemotactic or chemokinetic effect on SMC. The migration stimulated by PDGF was inhibited by STA₂ dose-dependently (Fig. 1).

STA₂ inhibited the proliferation of SMC in DMEM supplemented with 10% calf serum dose-dependently (Fig. 2).

STA₂ did not affect cell viability by the trypan blue dye exclusion test up to 10⁻⁵ M, after 2-hour incubation. STA₂ stimulated the release of LDH only in 10⁻⁵ M, and the lower concentrations of STA₂ did not injure SMC (data not shown).

STA₂ stimulated the release of PGI₂ from SMC; however, the effect was not inhibited by a TXA₂ receptor antagonist, ONO-3708, but rather increased by it (Fig. 3). ONO-3708 itself had no effect on the release of PGI₂.

Discussion

It has been reported that PDGF stimulates chemotaxis and proliferation of vascular SMC. In the present study, we showed that STA₂ inhibited the migration and proliferation of SMC dose-dependently. However, unlike PDGF, the half life time of TXA₂ is short and its effect will be limited to the localized small area around the thrombus. STA₂ injured SMC only in a high concentration (10⁻⁵ M).

It is interesting that SMC increased the PGI₂ release, when exposed to STA₂, probably as the response to the stimulation or injury by STA₂. Moreover, the effect by STA₂ was not inhibited by

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** Department of Geriatrics, Nagoya University School of Medicine, Nagoya 466, Japan

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Fig. 1 The effect of STA₂ on PDGF-stimulated SMC migration. 45 μ l of 3×10^5 /ml SMC suspended in DMEM containing 0.5% bovine serum albumin with or without STA₂ were incubated in the upper compartment for 6 hours. The lower compartment contained 25 μ l of the medium with PDGF except for the control. Mean $\pm$ S.D. (n=8)

Fig. 2 The effect of STA₂ on growth curves of SMC in DMEM supplemented with 10% calf serum. The method was described in details previously¹⁾. Cells were incubated in the medium with or without STA₂ for 10 days. Cell number was counted by a hemocytometer. Each point is the mean of 3 dishes.

TXA₂ receptor antagonist, ONO-3708. It may be conceivable that STA₂ induces PGI₂ release not by stimulating TXA₂ receptors of SMC which are

Fig. 3 The effect of STA₂ and ONO-3708 on PGI₂ release by SMC. SMC were preincubated in DMEM in the presence (shaded columns) or in the absence (open columns) of ONO-3708 for 30 min. After that, SMC were incubated in the new medium with or without STA₂ for more 30 min, and the released PGI₂ was assayed as described. Each column is the mean of 3 determinations.

antagonized by ONO-3708, but by CA²⁺ ionophore-like activity of STA₂ or by stimulating other receptors than those antagonized by ONO-3708. However, the mechanism remains to be known.

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References

- 1) Kuzuya, F., et al.: Effect of the calcium blocker, lanthanum, on bovine aortic smooth muscle cells in culture. *Artery*, **12**: 51 (1983).
- 2) Falk, W., et al.: A 48-well micro chemotaxis assembly for rapid and accurate measurement of leukocyte migration. *J. Immunol. Meth.*, **33**: 239 (1980).
- 3) Ohnishi, H., et al.: An evidence for "response to injury" hypothesis. *Life Sci.*, **31**: 2595 (1982).
- 4) Naito, M., et al.: An analog of thromboxane A₂ (STA₂) stimulates prostacyclin synthesis by endothelial cells and smooth muscle cells in culture. *Igakunoayumi*, **134**: 187 (1985) (in Japanese).

Summary

1) We studied the influences of a stable TXA_2 analogue, STA_2 , on cultured bovine aortic smooth muscle cells.

2) STA_2 (10^{-7} – 10^{-5} M) inhibited the PDGF-induced migration of SMC dose-dependently.

3) STA_2 (10^{-8} – 10^{-5} M) inhibited the proliferation of SMC dose-dependently.

4) STA_2 injured SMC only in a high concentration (10^{-5} M) when the damage was estimated by

LDH release.

5) STA_2 did not affect the viability of SMC examined by the trypan blue exclusion test.

6) STA_2 stimulated PGI_2 release of SMC; however, the effect of STA_2 was not suppressed by a TXA_2 receptor antagonist, ONO-3708 .

Key words: thromboxane A_2 , STA_2 , smooth muscle cell, migration, prostacyclin.