ELSEVIER

European Journal of Pharmacology 281 (1995) 205–207

ejp

Short communication

# Early administration of YT-146, an adenosine $A_2$ receptor agonist, inhibits neointimal thickening after rat femoral artery endothelium injury

Yoshiharu Takiguchi *, Mitsuyo Nagano, Yasuhiko Ikeda, Mitsuyoshi Nakashima

*Department of Pharmacology, Hamamatsu University School of Medicine, 3600 Handa-cho, Hamamatsu 431-31, Japan*

Received 16 March 1995; revised 29 May 1995; accepted 2 June 1995

**Abstract**

Adenosine is known to inhibit vascular smooth muscle cell proliferation in vitro via adenosine $A_2$ receptor activation. We tested the inhibitory effect of an adenosine $A_2$ receptor agonist, 2-octynyladenosine (YT-146), on in vivo intimal thickening following a photochemically induced injury of the endothelium of rat femoral artery. YT-146 (1 mg/kg/day) was administered s.c. for the first 3, 7 or 28 days after the injury. YT-146 significantly decreased neointimal area and the ratio of intima to medial area measured 28 days after the injury, regardless of the duration of administration. These results suggest that YT-146 may inhibit the early events of neointimal formation. The effect is long lasting and is not reversed even if YT-146 is stopped after a short course of administration.

*Keywords:* Adenosine $A_2$ receptor agonist; YT-146; Neointimal thickening; (Rat)

## 1. Introduction

Vascular endothelial injury is followed by platelet adhesion to the vessel wall and thrombus formation at the site of injury. Subsequently, vascular smooth muscle cells may proliferate, leading to intimal thickening. Such events can promote restenosis following coronary angioplasty and recanalization (Karas et al., 1991). Cyclic AMP has been reported to inhibit vascular smooth muscle cell growth. Thus agents that elevate (Loesberg et al., 1985; Shirotani et al., 1991; Souness et al., 1992) or mimic cyclic AMP (Southgate and Newby, 1990) inhibit the proliferation of vascular smooth muscle cells.

Adenosine induces changes in cyclic AMP production and modifies vascular smooth muscle cell proliferation through activation of two receptor subtypes, known as $A_1$ and $A_2$, which mediate inhibition and stimulation of adenylate cyclase, respectively. Cellular proliferation in vitro was inhibited by adenosine $A_2$ receptor activation, an effect attributable to an increase in cyclic AMP production (Jonzon et al., 1985). Therefore, selective adenosine $A_2$ receptor agonists are expected to have a beneficial effect in controlling intimal thickening, but such agents have not yet been investigated.

In the present study, we examined the ability of a potent selective adenosine $A_2$ receptor agonist, 2-octynyladenosine (YT-146), which has a 17-fold higher affinity for adenosine $A_2$ than for adenosine $A_1$ receptors (Abiru et al., 1991), to reduce neointimal thickening in our newly developed rat model of femoral artery thrombosis (Hirata et al., 1994). This model uses the photochemical reaction between rose bengal and green light to cause endothelial injury, which unlike the balloon injury model does not have any mechanical effect on the media (Matsuno et al., 1991).

## 2. Materials and methods

### 2.1. Animal model

Male Wistar rats weighing 250–280 g were anesthetized with sodium pentobarbital (50 mg/kg i.p.).

* Tel. 053 435 2271, fax 053 435 2270.

0014-2999/95/$09.50 © 1995 Elsevier Science B.V. All rights reserved
*SSDI* 0014-2999(95)00334-7

Table 1
Neointimal formation in the rat femoral artery after photochemically induced injury

| | Irradiated artery | Non-irradiated artery |
|---|---|---|
| Intimal area ($\times 10^{-2}$ mm$^2$) | 3.32 ± 0.30 | n.m |
| Medial area ($\times 10^{-2}$ mm$^2$) | 9.66 ± 0.44 | 9.87 ± 0.48 |

Results are expressed as means ± S.E.M. derived from 9 animals. n.m: not measurable.

The procedure to induce a transluminal thrombosis following endothelial injury in the femoral artery has been described in detail elsewhere (Matsuno et al., 1991). A part of the right femoral artery was carefully separated and a pulsed Doppler probe (PDV-20, Crystal Biotech America, USA) was placed on the vessel. Green light (wavelength 540 nm) irradiation was achieved by using a xenon lamp irradiation apparatus (L4887, Hamamatsu Photonics, Japan). The light was directed by an optic fiber positioned about 5 mm above a segment of the femoral artery proximal to the flow probe. Under irradiation, the photosensitizer dye, rose bengal (Sigma, USA) was injected (15 mg/kg) via the jugular vein. Light exposure was continued until the blood flow stopped due to thrombotic occlusion of the vessel. The time required to produce occlusion was about 10 min. The thrombotic occlusion was followed by spontaneous reperfusion within the first 24 h.

### 2.2. Measurement of intimal thickness

Intimal thickening was measured 28 days after the injury caused by thrombotic occlusion and spontaneous reflow. The irradiated (right) and non-irradiated control (left) segments of the femoral artery were fixed by infusion with 1% paraformaldehyde and 2% glutaraldehyde in 0.1 M phosphate buffered saline, pH 7.4. Then, both arteries were removed and fixed further by immersion in the same fixative. These specimens were sectioned transversely and stained with hematoxylin and eosin. The intimal and medial areas were identified by using a computer analysis system (Videoplane, Germany).

### 2.3. Drug treatment

YT-146 (Toa Eiyo, Japan) was administered s.c., at 1 mg/kg/day, using an osmotic pump (Alzet, USA) for the first 3, 7 or 28 days after thrombus formation.

### 2.4. Statistical analysis

All data are expressed as the means ± S.E.M. Comparison between the right and the left arteries was performed using paired $t$-test. The effects of YT-146 on intimal and medial areas were statistically analyzed using Dunnett's multiple range test after an analysis of variance. Results were considered significantly different if $P < 0.05$.

## 3. Results

The effect of the photochemical injury on the intimal and medial areas in the rat femoral artery is shown in Table 1. Intimal thickening was observed in all cases 28 days after the injury whereas the medial area of the irradiated artery was not different from that of the non-irradiated contralateral artery. Administration of YT-146 (1 mg/kg/day) significantly decreased the intimal area, without affecting the medial area, as compared with the untreated group (Fig. 1). Therefore, the ratio of intimal to medial area was significantly reduced in the YT-146-treated group. The inhibitory effect of YT-146 on neointima formation was observed regardless of the duration of administration after the

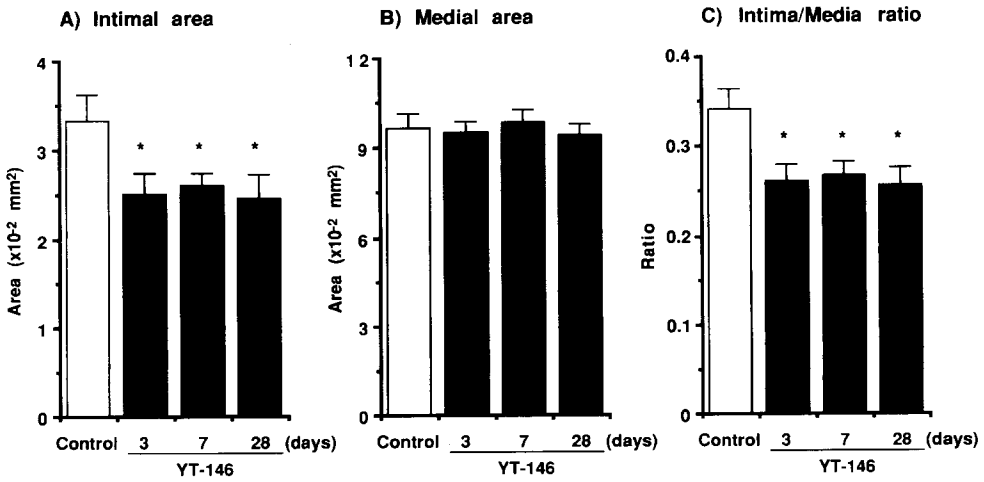


Fig. 1. Effect of YT-146 on intimal thickening in the rat femoral artery. YT-146, 1 mg/kg/day was administered s.c. using an osmotic pump for 3, 7 and 28 days after injury. Results are expressed as means ± S.E.M. derived from 9 animals. * $P < 0.05$ vs. control.

injury. Treatment for the first 3 and 7 days with YT-146 caused a significant reduction in intimal area, to the same extent as seen in the 28-day-treatment group.

## 4. Discussion

Vascular intimal thickening was inhibited by the new adenosine $A_2$ receptor agonist, YT-146. It was unexpected for us to see a 3-day or 7-day infusion to be as effective as a 28-day infusion in suppressing intimal thickening. The results suggest that adenosine, acting at adenosine $A_2$ receptors, may have an important suppressive role in the early response to injury in vivo.

The inhibitory effect of YT-146 on injury-induced intimal thickening may be a direct inhibitory effect on vascular smooth muscle cell proliferation. Adenosine has been reported to inhibit vascular smooth muscle cell proliferation by activation of adenosine $A_2$ receptors, an effect attributable to intracellular cyclic AMP elevation (Jonzon et al., 1985). Although the underlying mechanism of growth inhibition by cyclic AMP remains unknown, elevation of cyclic AMP at the $G_1$ phase of the cell cycle is necessary to inhibit proliferation, suggesting that it inhibits the progression from the $G_1$ to S phase (Loesberg et al., 1985; Fukumoto et al., 1988). In this study, we found that 3 days of treatment with YT-146 was effective. It is considered that the inhibition by YT-146 at the early stage of cell proliferation in response to injury leads to suppression of neointimal formation thereafter. This hypothesis is consistent with the work of Clowes and Clowes (1986) who have reported that the first 3 days of treatment with heparin, which inhibits the early entry of cells into S phase, produced a long-term reduction in vascular smooth muscle cell mass, resulting in inhibition of intimal thickening in the injured carotid artery.

Another possibility for the effect of YT-146 is inhibition of the early inflammatory reaction after injury. The early response to vascular injury is characterized by migration of inflammatory cells, including leukocytes and platelets, to the damaged vessel wall. These cells serve as a source of free radicals and growth factors such as platelet-derived growth factor, which may provide the stimulus for vascular smooth muscle cell migration and proliferation. Adenosine has been reported to have antiinflammatory and antiplatelet effects through adenosine $A_2$ receptors (Kogi et al., 1991; Cronstein, 1991).

In conclusion, activation of adenosine $A_2$ receptors may have a suppressive effect on vascular intimal thickening in injured artery in vivo.

## Acknowledgements

We wish to thank Dr. A.R. Saniabadi at the Japan Immunoresearch Laboratories, Takasaki for his critical reading of the manuscript. This study was supported by Grant-in Aid for Scientific Research 07672459 from the Ministry of Education, Science and Culture in Japan.

## References

Abiru, T., T. Yamaguchi, Y. Watanabe, K. Kogi, K. Aihara and A. Matsuda, 1991, The antihypertensive effect of 2-alkynyladenosines and their selective affinity for adenosine $A_2$ receptors, Eur. J. Pharmacol. 196, 69.

Clowes, A.W. and M.M. Clowes, 1986, Kinetics of cellular proliferation after arterial injury. IV. Heparin inhibits rat smooth muscle mitogenesis and migration, Circ. Res. 58, 839.

Cronstein, B.N., 1991, Adenosine is an autacoid of inflammation: effects of adenosine on neutrophil function, in: Role of Adenosine and Adenine Nucleotides in the Biological System, eds. S. Imai and M. Nakazawa (Elsevier, Amsterdam) p. 515.

Fukumoto, Y., Y. Kawahara, K. Kariya, S. Araki, H. Fukuzaki and Y. Takai, 1988, Independent inhibition of DNA synthesis by protein kinase C, cyclic AMP and interferon $\alpha/\beta$ in rabbit aortic smooth muscle cells, Biochem. Biophys. Res. Commun. 157, 337.

Hirata, Y., K. Umemura, K. Kondoh, T. Uematsu and M. Nakashima, 1994, Experimental intimal thickening studies using the photochemically induced thrombosis model in the guinea-pig femoral artery, Atherosclerosis 107, 117.

Jonzon, B., J. Nilsson and B.B. Fredholm, 1985, Adenosine receptor-mediated changes in cyclic AMP production and DNA synthesis in cultured arterial smooth muscle cells, J. Cell. Physiol. 124, 451.

Karas, S.P., E.C. Santoian and M.B. Gravanis, 1991, Restenosis following coronary angioplasty, Clin. Cardiol. 14, 791.

Kogi, K., T. Uchibori, K. Aihara, T. Yamaguchi and T. Abiru, 1991, Pharmacological profile of the 2-alkynyladenosine derivative 2-octynyladenosine (YT-146) in the cardiovascular system, Jpn. J. Pharmacol. 57, 153.

Loesberg, C., R. Van Wijk, J. Zandbergen, W.G. Van Aken, J.A. Van Mourik and P.G. De Groot, 1985, Cell cycle-dependent inhibition of human vascular smooth muscle cell proliferation by prostaglandin $E_1$, Exp. Cell Res. 160, 117.

Matsuno, H., T. Uematsu, S. Nagashima and M. Nakashima, 1991, Photochemically-induced thrombosis model in rat femoral artery and evaluation of effects of heparin and tissue-type plasminogen activator with use of this model, J. Pharmacol. Methods 25, 303.

Shirotani, M., Y. Yui, R. Hattori and C. Kawai, 1991, U-61,431F, a stable prostacyclin analogue, inhibits the proliferation of bovine vascular smooth muscle cells with little antiproliferative effect on endothelial cells, Prostaglandins 41, 97.

Souness, J.E., G.A. Hassall and D.P. Parrott, 1992, Inhibition of pig aortic smooth muscle cell DNA synthesis by selective type III and type IV cyclic AMP phosphodiesterase inhibitors, Biochem. Pharmacol. 44, 857.

Southgate, K. and A.C. Newby, 1990, Serum-induced proliferation of rabbit aortic smooth muscle cells from the contractile state is inhibited by 8-Br-cAMP but not 8-Br-cGMP, Atherosclerosis 82, 113.