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Short communication

Cilostazol suppresses intimal formation in dog grafted veins with reduction of angiotensin II-forming enzymes

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Abstract

Cilostazol prevents neointimal formation, but its mechanism has remained unclear. We investigated whether intimal formation in dog grafted veins is suppressed by cilostazol, and studied the effect of cilostazol on angiotensin II-forming enzymes. The external jugular vein was grafted to the carotid artery, and cilostazol (60 mg/kg/day) was administered orally. By 28 days after the surgery, the intimal cross-sectional area of the grafted vein was reduced to 16.7% by treatment of cilostazol, and the activities of angiotensin II-forming enzymes were suppressed significantly. The inhibitory effect of cilostazol in intimal formation may be dependent on inhibition of angiotensin II-forming enzymes.

Keywords: Phosphodiesterase; Angiotensin II; Grafting; Stenosis

1. Introduction

In coronary bypass surgery, the internal thoracic artery and saphenous vein are used as artery bypass candidates. However, the poor outcome of the saphenous vein used as the bypass candidate is well known (Lytle et al., 1992; Motwani and Topol, 1998). The mechanism of early occlusions of the saphenous vein has been unclear and drugs that prevent vascular proliferation are in great demand.

Cilostazol is a potent antiplatelet agent that selectively inhibits phosphodiesterase-3 in platelets and vascular smooth muscle cells, resulting in inhibition of platelet-aggregation and vasodilation (Tanaka et al., 1998). Cilostazol used after coronary stenting is associated with a low rate of stent thrombosis (Ochiai et al., 1997). Moreover, cilostazol appears to have an inhibitory effect on intimal proliferation after directional coronary stenting and percutaneous transluminal coronary angioplasty (Sekiya et al., 1998; Tsuchikane et al., 1999). However, it has been unclear why cilostazol prevents vascular proliferation.

Angiotensin II plays an important role in the proliferation of vascular tissues. An angiotensin-converting enzyme inhibitor was effective in preventing this proliferation in rat grafted vessels (Roux et al., 1991). On the other hand, in a baboon model, the vascular proliferation in the grafted veins was not suppressed by an angiotensin-converting enzyme inhibitor (Hanson et al., 1991). Baboon vascular tissues, but not rat vascular tissues, contain chymase as an angiotensin II-forming enzyme (Chandrasekharan et al., 1996), and an angiotensin-converting enzyme inhibitor may not prevent vascular proliferation in this species. We demonstrated that both angiotensin II-forming enzyme and chymase activities were increased in dog grafted veins and an angiotensin AT_1 receptor antagonist prevented this vascular proliferation (Yuda et al., 2000). Angiotensin II formed by angiotensin-converting enzyme or chymase may play a crucial role in vascular proliferation in grafted veins. In this report, we investigated whether cilostazol could prevent vascular proliferation in dog grafted veins, and studied the effect of cilostazol on angiotensin II-forming enzymes.

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2. Materials and methods

2.1. Animals

Eight dogs were obtained from Japan SLC (Shizuoka, Japan). In the cilostazol-treated group, the drug (30 mg/kg) was administered orally twice daily from 7 days before the surgery to 28 days after it. The animals were anesthetized with sodium pentobarbital (35 mg/kg, i.v.), and the right external jugular vein was grafted to the ipsilaterial carotid artery (Takai et al., 2000). The experimental procedures for animals were in accordance with the Guide for the Care and Use of Laboratory Animals (Animal Research Laboratory, Osaka Medical College).

2.2. Preparation of vascular tissue

At 28 days after the surgery, the veins were removed, and cut in half. One segment was used for the measurement of cross-sectional area, and the other segment was used for assay of the angiotensin II-forming enzymes.

2.3. Histological analysis of vascular tissue

The vessel segments were fixed in 10% neutral buffered formalin, embedded in paraffin and cut into 5-μm thick sections. These sections were stained with elastica-van Gieson, and the cross-sectional areas of intima and media were quantified with an image analysis system (VM-30, Olympus Optical, Tokyo, Japan).

2.4. Measurement of angiotensin-converting enzyme and chymase activities

The veins were homogenized in 20 mM phosphate buffer, pH 7.4. The homogenate was centrifuged at 20,000 $\times g$ for 30 min. The pellets were homogenized in 10 mM phosphate buffer, pH 7.4, containing 2 M KCl and 0.1% Nonidet P-40. The homogenate was centrifuged at 20,000 $\times g$ for 30 min. The supernatant was used for the measurements of angiotensin-converting enzyme and chymase activities.

The angiotensin-converting enzyme activity was measured by incubating the tissue extracts at 37°C with 5 mM hippuryl-His-Leu of a synthetic substrate in 100 mM phosphate buffer, pH 8.3, containing 800 mM NaCl (Takai et al., 2000). One unit was defined as the amount of enzyme that cleaved 1 μmol hippuric acid/min.

The chymase activity was measured by incubating tissue extracts for 30 min at 37°C with 770 μM angiotensin I in 150 mM borax-borate buffer, pH 8.5, containing 8 mM dipyridyl, 770 μM diisopropyl phosphorofluoridate and 5 mM ethylenediaminetetraacetic acid (Takai et al., 2000). One unit was defined as the amount of enzyme that formed 1 μmol angiotensin II/min.

2.5. Statistics

All experiments were conducted with four dogs per group. Statistical analysis was conducted by analysis of variance (ANOVA) followed by a Student t test. Values of $P < 0.05$ were considered significant. Data are expressed as mean ± standard error of the mean (S.E.M.).

3. Results

3.1. Histological analysis

The chymase inhibitor suppressed the proliferation of the intimal cross-sectional area, but not of the medial cross-sectional area (Fig. 1). In the placebo-treated group, the intimal cross-sectional area of the grafted vein was 3.38 ± 0.34 mm^2, but that of the symmetrical vein could hardly be measured. The mean intimal cross-sectional area of the placebo-treated animals was regarded as 100%, while the area of cilostazol-treated animals was reduced to 16.7%.

3.2. Activities of angiotensin-converting enzyme and chymase

Fig. 2 shows the activities of angiotensin-converting enzyme and chymase in vessels treated with placebo and cilostazol. In the placebo-treated group, the chymase activity in the grafted veins (11.7 ± 1.81 mU/mg protein) was increased significantly when compared with the value (0.75 ± 0.07 mU/mg protein) in the control veins. The angiotensin-converting enzyme activities in the grafted and control veins were 4.60 ± 0.48 and 2.16 ± 0.23 mU/mg protein, respectively, and this difference was also significant. In the cilostazol-treated group, the chymase and angiotensin-converting enzyme activities were 0.97 ± 0.12

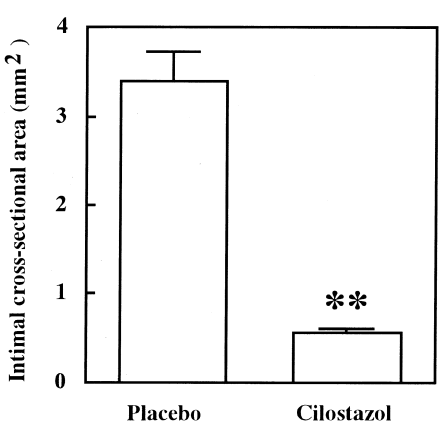

Fig. 1. Effects of cilostazol (60 mg/kg/day) on the intimal cross-sectional area in the grafted veins 28 days after the surgery. $^{**}P < 0.01$ vs. grafted veins in placebo-treated group ($N = 4$).

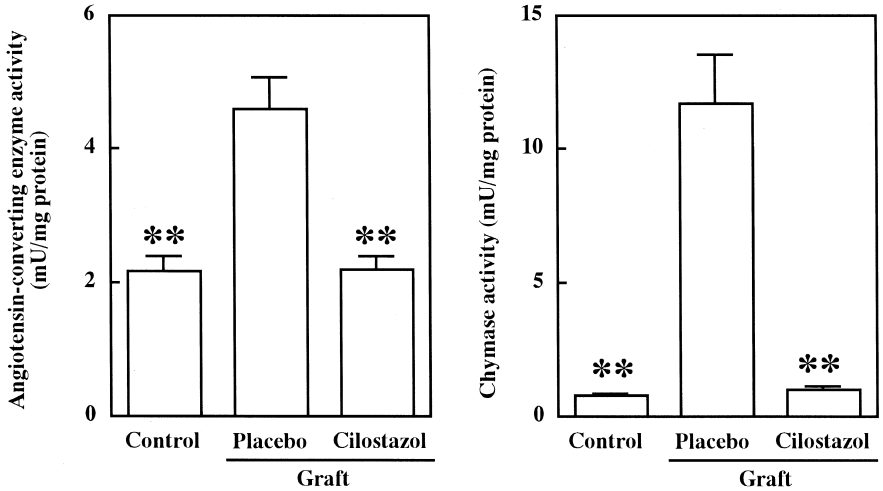

Fig. 2. Effects of cilostazol (60 mg/kg/day) on angiotensin-converting enzyme and chymase activities in the grafted veins 28 days after the surgery. ** $P < 0.01$ vs. grafted veins in placebo-treated group ($N = 4$).

and 2.19 ± 0.20 mU/mg protein, respectively, thus strongly reduced in the grafted veins.

4. Discussion

In the present study, we demonstrated for the first time that cilostazol suppressed vascular proliferation in grafted veins in dog. Furthermore, surprisingly, cilostazol also strongly suppressed the activities of the angiotensin II-forming enzymes, chymase and angiotensin-converting enzyme, in the grafted veins. Stenosis of the coronary arteries is mainly treated by surgical therapies such as grafts or by intracoronary therapies such as percutaneous transluminal coronary angioplasty. In rat balloon-injury model, an angiotensin-converting enzyme inhibitor was effective in preventing the proliferation of injured artery (Powell et al., 1989). Based on these reports, it was studied whether an angiotensin-converting enzyme inhibitor could suppress human vascular restenosis after percutaneous transluminal coronary angioplasty, but the result was negative (MERCATOR Study Group, 1992). We reported previously that the activities of both angiotensin-converting enzyme and chymase in dog arteries injured by a balloon catheter were increased compared with those in uninjured arteries (Miyazaki et al., 1999). In this model, an angiotensin AT_1 receptor antagonist was effective in preventing intimal formation of vessels after injury, but an angiotensin-converting enzyme inhibitor was ineffective (Miyazaki and Takai, 2000). Vascular tissues in human and dog contain chymase-dependent angiotensin II formation (Takai et al., 1999; Caughey et al., 2000) and in these species, angiotensin AT_1 receptor antagonist may be more effective in preventing the vascular proliferation than the angiotensin-converting enzyme inhibitor. Cilostazol suppressed both angiotensin-converting enzyme and chymase activities in grafted veins, and the effect of cilostazol may be dependent on its suppression of angiotensin II formation in grafted veins.

Cilostazol has been known as an antiplatelet drug, but aspirin could not suppress to intimal hyperplasia after percutaneous transluminal coronary angioplasty (Tsuchikane et al., 1999). This finding suggests that the effect of cilostazol in vascular proliferation does not depend on its antiplatelet effect. The mechanism whereby cilostazol reduces restenosis after percutaneous transluminal coronary angioplasty is thought to be primarily due to suppressing the migration of smooth muscle cells and increase of extracellular matrix (Tsuchikane et al., 1999). As an antiplatelet medication, cilostazol reduces the activation of platelet-derived growth factor, which induces migration of smooth muscle cells and extracellular matrix, from activated platelets. However, another antiplatelet drug, aspirin, did not suppress intimal hyperplasia in spite of inhibiting the activation of platelets (Tsuchikane et al., 1999). Cilostazol increases intracellular cAMP and decreases [^{3}H] thymidine uptake of smooth muscle cells (Pan et al., 1994). However, the mechanism by which an increase in the concentration of cAMP results in the inhibition of cell growth has been unclear. In this study, we demonstrated for the first time that cilostazol strongly suppressed the activities of angiotensin-converting enzyme and chymase in grafted veins. Angiotensin II is known to induce migration of smooth muscle cells and to increase extracellular matrix (Kim and Iwao, 2000). Cilostazol suppresses the angiotensin II formation from both angiotensin-converting enzyme and chymase, and may reduce the migration of smooth muscle cells and the induction of extracellular matrix, resulting in the prevention of vascular proliferation.

In conclusion, cilostazol prevents vascular proliferation in grafted veins, and this prevention may be dependent on its inhibition of angiotensin II-forming enzymes.

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