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Tranilast suppresses the vascular intimal hyperplasia after balloon injury in rabbits fed on a high-cholesterol diet

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Abstract

Intimal hyperplasia is a serious problem after percutaneous transluminal coronary angioplasty. In this study, we assessed the effect of tranilast on vascular intimal hyperplasia after balloon injury in rabbits fed on a high-cholesterol diet. In this animal model, intimal hyperplasia more severe than that in rabbits fed on a normal diet was observed. In addition, medial thickening and lipid deposits in both media and intima were also noted. These findings indicate that balloon injury caused intimal and medial hyperplasia and that this hyperplasia was accelerated by the high cholesterol load. Tranilast (300 mg/kg) significantly decreased the intimal area, medial area, and stenosis ratio, and increased the luminal/total area ratio, in the cholesterol-fed rabbits. These results suggest that tranilast may be useful for prevention of restenosis after percutaneous transluminal coronary angioplasty of patients, including those with a clinical risk of hypercholesterolemia.

Keywords: Tranilast; Hyperplasia, intimal; Balloon injury; Cholesterol, high

1. Introduction

Percutaneous transluminal coronary angioplasty is one of the widely used therapies for patients with ischemic coronary artery disease; however, 30–40% of such patients suffer from vascular restenosis within 6 months after the operation (Serruys et al., 1988; Kaltenbach et al., 1985). The restenosis results from the migration and proliferation of vascular smooth muscle cells and the accumulation of excessive extracellular matrix produced by these cells (Clowes and Schwartz, 1985).

Though many attempts have been made to develop drugs to prevent the restenosis, no drug has yet been used clinically. Tranilast is a drug that is used not only for patients with bronchial asthma and allergic rhinitis but also for those with proliferative diseases such as keloid and hypertrophic scars. Recently, we reported that tranilast suppressed the vascular intimal hyperplasia observed after balloon injury via inhibition of vascular smooth muscle cell migration and proliferation, and reduced the accumulation of excessive extracellular matrix produced by vascular smooth muscle cells, in rabbits fed on a normal diet (Fukuyama et al., 1996). However, it has been reported that hypercholesterolemia enhances intimal hyperplasia after balloon injury (Ferns et al., 1992). Also, hypercholesterolemia is known as one of the risk factors of coronary artery disease and atherosclerosis. In this study, therefore, we assessed the effect of tranilast on the vascular intimal hyperplasia occurring after balloon injury in rabbits fed on a high-cholesterol diet.

2. Materials and methods

2.1. Animals

Japanese White male rabbits (body weight approx. 3 kg) purchased from KEARI (Osaka, Japan) were used. These rabbits were housed individually from 1 week before the experiment in a temperature- (23 ± 2°C) and humidity (55 ± 5%)-controlled room, and were fed throughout the experimental period on a high-cholesterol diet (containing 1% cholesterol and 5% lard, RC-4) purchased from Oriental Yeast (Tokyo, Japan).

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2.2. Balloon injury model

Rabbits were anesthetized with 30 mg/kg of sodium pentobarbital injected intravenously. Bilateral common carotid arteries were exposed and dissected from the surrounding tissues. An arterial embolectomy catheter (2Fr, Baxter Healthcare, Santa Ana, CA, USA) was inserted about 5 cm into the right common carotid artery through an incision made in the wall of the vessel. The balloon was inflated to a pressure of 1200 mmHg, and the intraluminal surface of the artery was rubbed by one pullback of the balloon. After this rubbing, the balloon catheter was with-

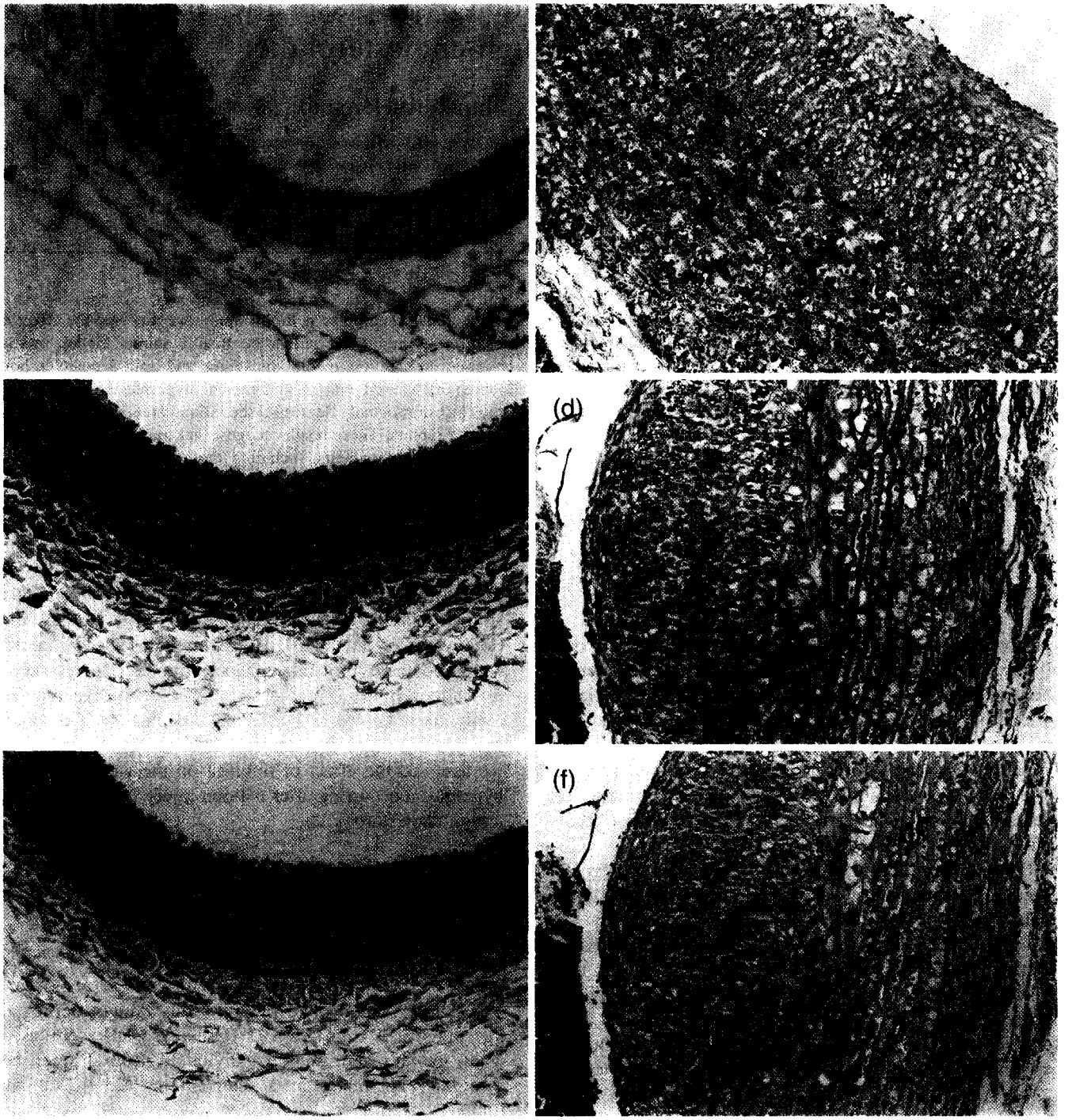

Fig. 1. Photomicrographs of Oil Red O- (a,b), azan- (c,d), and hematoxylin-eosin (e,f)-stained common carotid arteries after ballon injury in rabbits fed on a high-cholesterol diet. Arteries were removed on the 28th day after the balloon injury. a, c, and e: Artery from a sham-operated rabbit. b, d, and f: Artery from a balloon-injured rabbit. Original magnification: a and b, ×40; c, d, e, and f, ×50.

drawn from the vessel, and the incision on the wall of vessel was sutured. The left common carotid artery was used for a sham operation.

2.3. Histological observation

The rabbits were killed by exsanguination under anesthesia at 28 days after the balloon injury. Right and left common carotid arteries were removed and fixed in a 10% neutral solution of formaldehyde, and each fixed vessel was cut into two segments. One segment was embedded in paraffin, and transverse sections were cut from these blocks. For histological examination, sections were stained by using hematoxylin-eosin, azan, and Elastica-Van Gieson. The other fixed segment was frozen, sectioned with a cryostat, and then stained with Oil Red O.

2.4. Morphometric analysis

The intimal, medial, and luminal areas of the sections stained by the Elastica-Van Gieson method were measured with a morphometric analyzer (Lusex III, Nikon, Tokyo, Japan). Results are expressed as intimal and medial areas, stenosis ratio, and luminal/total area ratio. The stenosis ratio and the luminal/total area ratio are expressed as follows: stenosis ratio (%) = (intimal area × 100)/(intimal area + luminal area); luminal/total area ratio (%) = (luminal area × 100)/(intimal + medial + luminal area).

2.5. Drugs

Tranilast, *N*-(3,4-demethoxycinnamoyl) anthranilic acid, was suspended in 0.5% carboxymethylcellulose solution and was administered perorally once a day, starting the day after the operation. In the sham operation and the control groups, 0.5% carboxymethylcellulose solution was administered. The doses of tranilast were selected according to the plasma concentration that showed efficacy in a rat granuloma model (Suzawa et al., 1992c), and the doses in clinical use. The plasma concentration of tranilast after administration of 100 mg/kg in rats and 200 mg/body in humans was nearly equal to that after administration of 200–300 mg/kg in rabbits (data not shown), so we used the doses of tranilast from 100 to 300 mg/kg in this study.

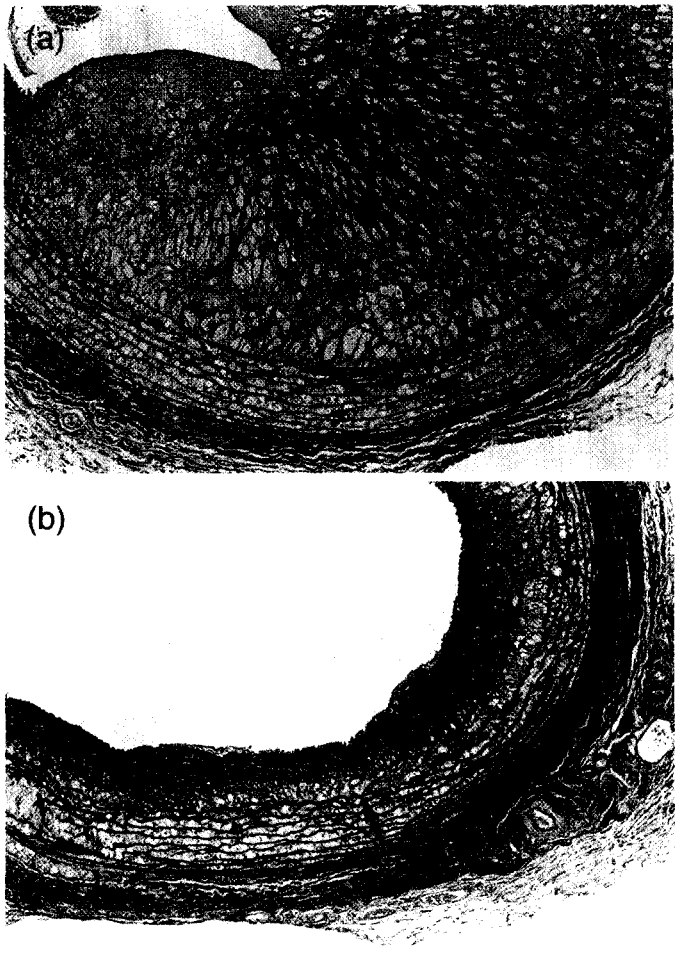

Fig. 2. Typical photomicrographs of Elastica-Van Gieson-stained common carotid arteries after balloon injury in rabbits fed on a high-cholesterol diet. a: Injured artery from a control rabbit. b: Injured artery from a tranilast (300 mg/kg)-treated rabbit. Original magnification: a and b, ×20.

2.6. Data analysis

Statistical analysis was performed by analysis of variance (ANOVA) and Fisher's test using the Stad View 4.0 software program (Abacus Concepts, Berkeley, CA, USA). Data are presented as the means ± S.E.

3. Results

3.1. Histological findings

Typical histological findings for vessels taken on the 28th day after balloon injury are shown in Fig. 1. In the sham-operated common carotid artery, no noticeable changes could be detected (Fig. 1a, c and e). After the balloon injury, lipid deposition was observed in both intima and media (Fig. 1b). A neointima composed of elastic and collagen fibers was found (Fig. 1d), and degenerated cells were observed in the media after the balloon injury (Fig. 1f).

3.2. Effects of tranilast after balloon injury

Typical histological findings are shown in Fig. 2. On the 28th day after balloon injury, marked intimal thickening was observed in the control rabbits (Fig. 2a). Treatment with tranilast (300 mg/kg) decreased this intimal thickening (Fig. 2b). The statistical results of morphometric analysis are shown in Figs. 3–5. In the control rabbits fed on a high-cholesterol diet, the intimal area of the injured right common carotid arteries was 1.15 ± 0.07 mm^2 (left panel); the damage was more extensive than that in rabbits fed on the normal diet (right panel). Tranilast (300 mg/kg) significantly decreased this area to 0.80 ± 0.07 mm^2 (Fig. 3). The medial area in control rabbits fed on the high-cholesterol diet also increased (left panel), but

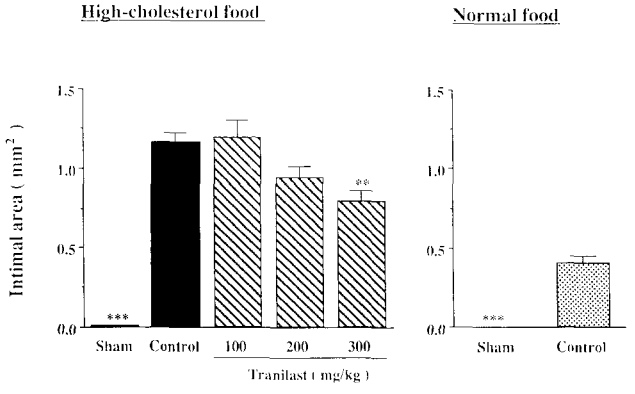

Fig. 3. Effect of tranilast on the intimal area in common carotid arteries after balloon injury in cholesterol-fed rabbits (left panel) and in rabbits fed on a normal diet (right panel). The arteries were removed on the 28th day after injury. Data are presented as the means ± S.E. for 11–17 animals in the left panel and for 8–10 animals in the right panel.

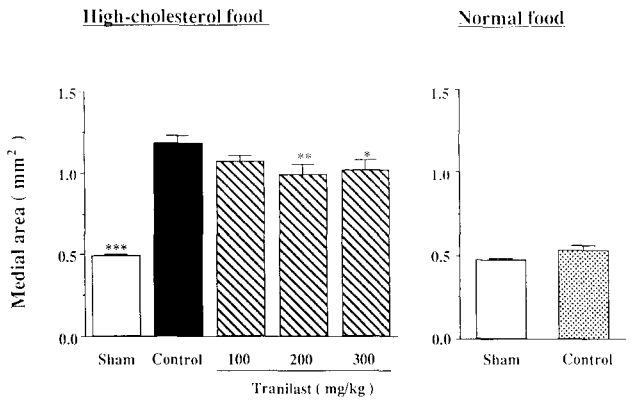

Fig. 4. Effect of tranilast on the medial area in common carotid arteries after balloon injury in cholesterol-fed rabbits (left panel) and in rabbits fed on a normal diet (right panel). The arteries were removed on the 28th day after injury. Data are presented as the means ± S.E. for 11–17 animals in the left panel and for 8–10 animals in the right panel. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$, as compared with the control.

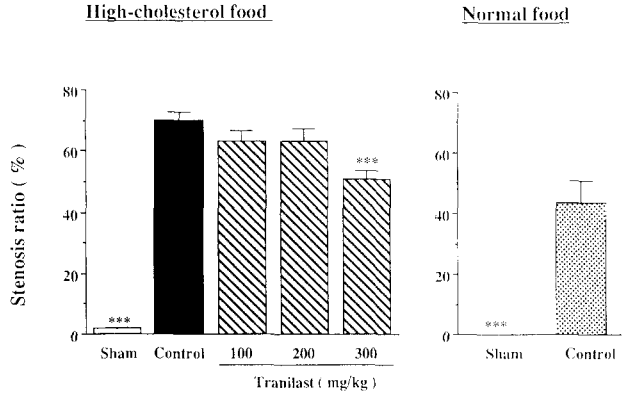

Fig. 5. Effect of tranilast on the stenosis ratio in common carotid arteries after balloon injury in cholesterol-fed rabbits (left panel) and in rabbits fed on a normal diet (right panel). The arteries were removed on the 28th day after injury. Data are presented as the means ± S.E. for 11–17 animals in the left panel and for 8–10 animals in the right panel. *** $P < 0.001$, as compared with the control.

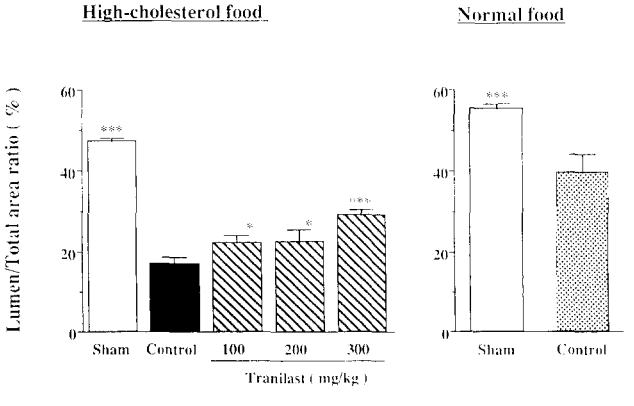

Fig. 6. Effect of tranilast on the luminal/total area ratio in common carotid arteries after balloon injury in cholesterol-fed rabbits (left panel) and in rabbits fed on a normal diet (right panel). The arteries were removed on the 28th day after injury. Data are presented as the means ± S.E. for 11–17 animals in the left panel and for 8–10 animals in the right panel. * $P < 0.05$, and *** $P < 0.001$, as compared with the control.

this increase was not observed in rabbits fed on the normal diet (right panel). Tranilast (200 and 300 mg/kg) significantly suppressed this increase (Fig. 4). As to the stenosis ratio, the value for the control rabbits fed on the high-cholesterol diet was 70.0 ± 2.9%; it was higher than that in rabbits fed on the normal diet. Tranilast (300 mg/kg) significantly decreased the stenosis ratio to 50.6 ± 2.8% (Fig. 5). The luminal/total area ratio decreased in the hypercholesterolemic rabbits compared with that in the normocholesterolemic rabbits. Tranilast significantly suppressed this decrease (Fig. 6).

4. Discussion

Percutaneous transluminal coronary angioplasty is one of the widely used therapies for patients with coronary artery disease. However, vascular restenosis, which is caused by vascular intimal hyperplasia, is a serious problem and is a result of vascular injury caused by such an operation. Previously, we reported that tranilast inhibited the intimal thickening in rabbits fed on a normal diet via a direct effect on the vascular smooth muscle cells (Fukuyama et al., 1996). It is known that hypercholesterolemia is one of the risk factors of ischemic coronary artery disease and atherosclerosis. Furthermore, it is reported that intimal hyperplasia induced by balloon injury is more severe in hypercholesterolemic rabbits (Ferns et al., 1992). So, in this study, we assessed the effect of tranilast on intimal hyperplasia after balloon injury in rabbits fed on a high-cholesterol diet.

After endothelial injury such as that caused by a balloon catheter, platelets aggregate in the injured area. Then, certain cytokines, such as platelet-derived growth factor (PDGF), are released from the aggregated platelets and vascular smooth muscle cells, and these cytokines play important roles in the development of intimal hyperplasia (Bornfeldt et al., 1994; Mogami and Kojima, 1993; Tanaka et al., 1994). In addition, in rabbits fed on a high-cholesterol diet, many macrophages and foam cells are observed in the injured area, and these cells participate in the intimal hyperplasia (Stadius et al., 1994; Ferns et al., 1992; Liu et al., 1990). It is reported that macrophages and foam cells also release cytokines which induce intimal hyperplasia (Falcone et al., 1993; Ross, 1993).

In the animal model used in this study, marked intimal hyperplasia was observed. Moreover, medial hyperplasia, degenerated cells in the media, and lipid deposition in both the intima and media were also found. From these observations, we consider the animal model used in this study to be one of severe intimal and medial hyper-repair, in which hyperplasia is initiated by balloon injury and enhanced by lipid deposition.

The results of our assessment of the effects of tranilast in this animal model indicate that tranilast decreased the intimal area and the stenosis ratio. Furthermore, the drug increased the luminal/total area ratio. These results suggest that tranilast suppresses intimal hyperplasia and remodeling after balloon injury.

No anti-thrombotic effect of tranilast was seen in our earlier studies or in a study by others (Kikuchi et al., 1996). So, the effect of tranilast on intimal hyperplasia depends on the inhibition of the pathway subsequent to platelet aggregation. It is reported that tranilast inhibits the release of chemical mediators from mast cells and basophils (Koda et al., 1976; Komatsu et al., 1988), and the production of cytokines and oxygen free radicals from activated macrophages and neutrophils (Suzawa et al., 1992a,b,c). Recently, it was shown that tranilast inhibits vascular smooth muscle cell migration and proliferation, and collagen synthesis by vascular smooth muscle cell (Miyazawa et al., 1995; Tanaka et al., 1994), and that tranilast suppresses the intimal hyperplasia in spontaneously hypertensive rats and in rabbits fed on a normal diet via inhibition of this pathway (Kikuchi et al., 1996; Fukuyama et al., 1996). Tranilast decreased the serum cholesterol level, but only slightly (data not shown). And, lovastatin did not reduce the intimal hyperplasia in a clinical trial (Weintraub et al., 1994). These results indicate that lowering the cholesterol level does not prevent the restenosis and that the suppressive effect of tranilast on the intimal hyperplasia does not depend on lowering of the cholesterol level. Therefore, we suggest that tranilast suppresses intimal hyperplasia by inhibition of vascular smooth muscle cell migration and proliferation, and by reduction of the excessive accumulation of collagen produced by vascular smooth muscle cells in hypercholesterolemc animals, as it does in normocholesterolemic animals.

In this study, tranilast also inhibited the increase in the medial area. There are few reports considering medial thickening, so the pathway of medial hyperplasia is unclear. However, medial thickening was observed after balloon injury in pigs, and the authors speculated that such thickening may be important in human restenosis (Groves et al., 1995). Therefore, we speculate that the suppressive effect of tranilast on medial thickening may be a desirable effect for the prevention of restenosis. However, more experiments are needed to clarify this matter.

Some drugs, such as heparin (Clowes et al., 1991) and angiotensin-converting enzyme inhibitor (Osterrieder et al., 1991; Clozel et al., 1991), inhibit intimal hyperplasia in animal models, but have no effect in humans. In this study, we showed that tranilast suppressed the intimal hyperplasia caused by balloon injury in hyper-cholesterolemic rabbits in the same manner as in normocholesterolemic animals. Moreover, it has been reported that tranilast has a potent effect in preventing restenosis after percutaneous transluminal coronary angioplasty in a double-blind scale multicenter trial (The TREAT Study Investigators, 1994). These results indicate that tranilast suppresses intimal hyperplasia not only in animal models but also in humans, and suggest the possibility that tranilast may be useful to prevent

restenosis after percutaneous transluminal coronary angioplasty in both hyper- and normocholesterolemic patients.

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