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Neutrophil accumulation promotes intimal hyperplasia after photochemically induced arterial injury in mice

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Abstract

The role of leukocytes in the pathogenesis of coronary arterial disease has become a focus for clinical research. The aim of this study was to determine whether neutrophil accumulation would participate in the development of intimal hyperplasia after endothelial injury in mice, and whether D-*myo*-inositol hexakisphosphate (phytic acid) which inhibits the binding of L- and P-selectin to sialyl Lewis^X could inhibit the development of intimal hyperplasia. Endothelial injury was inflicted in one femoral artery via the photochemical reaction between systemically injected rose bengal and transillumination with green light (wavelength: 540 nm). Scanning electron microscopic observation at 3 days after the injury showed an increase in the number of leukocytes adhering to the injury site. Histological observation at 21 days showed that in the neutropenia group administered anti-neutrophil antibody and in the phytic acid-treated group the progression of intimal hyperplasia was significantly attenuated by comparison with the corresponding control groups.

These results suggest that neutrophil accumulation contributes to the initiation and/or development of intimal hyperplasia and L- and/or P-selectin may participate in their mechanisms.

Keywords: Leukocyte; Neutrophil; Intimal hyperplasia; Selectin; Phytic acid

1. Introduction

Percutaneous coronary intervention (PCI) is widely used as a therapy for occlusive coronary arterial disease. Despite improvements in technique such as the use of stents with antiplatelet agents or drug-eluting stents, restenosis occurs in ≈8% of patients within 6 months (Montalescot et al., 2001; Kastrati et al., 2005). Consequently, the identity of the factors responsible for the development of restenosis in patients subjected to PCI is a subject of considerable interest (Schwartz et al., 1995). Neumann et al. (1996) have reported (i) that the surface expression of macrophage antigen-1 (Mac-1) on neutrophils sampled downstream of the dilated coronary artery segment is significantly higher than that in prestenotic segments in patients who had undergone angioplasty, and (ii) that the expression of L-selectin on neutrophils is concomitantly decreased downstream of the dilated coronary artery segment by comparison with that seen in prestenotic segments. To judge from these results, neutrophils may play an important role in the initiation and/or development of intimal hyperplasia and restenosis.

In 1982, a postmortem study demonstrated an accumulation of leukocytes as well as of platelets at the site of vascular injury after angioplasty in humans (Block et al., 1982). Leukocyte accumulation at a site of inflammation is regulated by the level of their binding to the endothelium (Butcher, 1991), and the multistep process involved is initiated by a family of carbohydrate-binding adhesion molecules called selectins. In this process, leukocyte rolling is mediated by the

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molecular interaction of the selectin family [P-selectin (CD62P), endothelial E-selectin (CD62E), and leukocyte L-selectin (CD62L)] either with their principle carbohydrate ligand [sialyl Lewis^X (SLe^X)] or with other sialylated, fucosylated sugar chain structures in the glycoproteins and glycolipids expressed by a variety of cell types, including neutrophils, monocytes, and colon cancer cells (Carlos and Harlan, 1994). Leukocyte rolling appears to be followed sequentially by the interaction of the β_2 integrins, lymphocyte function-associated antigen-1 (LFA-1; CD11a/CD18) (Marlin and Springer, 1987), macrophage antigen-1 (Mac-1; CD11b/CD18) (Smith et al., 1989), and p150,95 (CD11c/CD18) (Blackford et al., 1996), on neutrophils with their counter receptors on endothelial cells [via intercellular adhesion molecules-1 and -2 (ICAM-1 and-2)] (Diamond et al., 1990; De Fougerolles et al., 1991). This is followed by firm adhesion of leukocytes to the endothelial surface and the transmigration of neutrophils to extravascular sites (Smith et al., 1988; Sligh et al., 1993).

Thus, adhesion molecules such as selectins and integrins play pivotal roles in inflammatory responses. However, little is known about the participation of leukocytes, particularly neutrophils, and their adhesion molecules, such as integrins and selectins, in intimal hyperplasia. D-*myo*-inositol hexakisphosphate (phytic acid) has been reported to inhibit (i) the binding of L- and P-selectin-Ig-fusion protein to immobilized bovine serum albumin-SLe^X and (ii) the neutrophil accumulation in the peritoneal cavity of mice that occurs after zymosan or thioglycollate injection (Cecconi et al., 1994). In the present study, we examined the effects of neutropenia (produced using anti-murine neutrophil antiserum) and phytic acid and on the intimal hyperplasia that follows a photochemically induced injury to the endothelium in the mouse femoral artery.

2. Materials and methods

2.1. Animals

Ninety-nine male ICR mice (Japan SLC, Shizuoka, Japan) weighing 23 to 28 g were used. The experiments in this study were conducted in accordance with institutional guidelines. The protocols were approved by the Animal Experiments Committee of Hamamatsu University School of Medicine.

2.2. Experiments involving endothelial injury

2.2.1. Infliction of endothelial injury

Seventy mice were anesthetized with sodium pentobarbital (80 mg/kg, i.p.) and body temperature was maintained at 37.5 °C throughout the experiment with the aid of a heating pad (K-module Model K-20; American Pharmseal Company, USA). The procedure used to induce a transluminal thrombosis in the femoral artery was the same as that described in detail elsewhere (with minor modifications) (Kikuchi et al., 1998). Briefly, the right femoral artery was exposed and the probe (1 mm diameter; Advance) of a laser Doppler flowmeter (ALF2100; Advance, Tokyo, Japan) was carefully placed onto the surface of the artery for monitoring blood flow, the probe signal being recorded continuously using a recorder (U-228; Nippon Denshi Kagaku, Kyoto, Japan). The jugular vein was cannulated with a polyethylene tube for the administration of rose bengal (Wako, Japan). Transillumination with green light (540 nm wavelength) was achieved by means of an L4487 irradiation apparatus (Hamamatsu Photonics, Japan), the irradiation being directed with an optic fiber positioned 5 mm away from the part of the right femoral artery proximal to the flow probe. After establishing baseline blood flow, irradiation was started and rose bengal at 20 mg/kg (i.v.) was injected over the next 5 min. Photo-irradiation was continued for 15 min, with the blood flow being monitored for 15 min from the start of the rose bengal injection. The formation of an occlusive thrombus was indicated by a complete cessation of blood flow. The time to achieve complete occlusion was about 10 min. After removal of the polyethylene tube, the jugular vein was ligated, and the surgical wound was closed. A thrombotic occlusion of this type is followed by spontaneous reperfusion within the first 24 h (Kikuchi et al., 1998).

2.2.2. Histopathological observation

Changes in the femoral arteries of a group of 6 of the mice that had received a photochemically induced endothelial injury were analyzed by scanning electron microscopy or light microscopy. At 1 or 3 days after the injury, mice were reanesthetized (sodium pentobarbital, 80 mg/kg, i.p.) and perfused at physiological pressure with saline followed by 1% paraformaldehyde and 2% glutaraldehyde in 0.01% phosphate-buffered saline (PBS). Segments of the irradiated and non-irradiated femoral arteries were then removed and kept in the same fixative buffer (pH 7.4) overnight at 4 °C. The specimens were then either prepared for scanning electron microscopy by the method of Muranaka et al. (1988) and examined with a Hitachi S-800 scanning electron microscope, or stained for light microscopy with hematoxylin and eosin or May–Grünwald–Giemsa.

2.2.3. Effect of anti-neutrophil antibody or phytic acid on intimal hyperplasia

Neutrophil depletion was induced by a procedure described in detail elsewhere (with minor modifications) (Hodes et al., 1974; Connolly et al., 1996). In the first series of experiments (24 mice), rabbit anti-mouse neutrophil antibody (0.3 ml of 1:30 solution) preadsorbed onto erythrocytes, or an identical volume of vehicle, was administered intraperitoneally 3 days before the initiation

of the photochemical reaction, and then once a day for 10 days. In another series of experiments (40 mice), phytic acid [10 mg (11 μmol) or 30 mg (33 μmol)/kg)], inositol [6 mg (33 μmol)/kg], or an identical volume of vehicle (5 ml/kg) was administered subcutaneously 1 h after the initiation of the photochemical reaction, and then once a day for 7 days. Intimal hyperplasia was measured as described below.

2.2.4. Measurement of intimal hyperplasia

Intimal hyperplasia was measured 21 days after the endothelial injury induced by the photochemical reaction described above. Briefly, the mice were reanesthetized (sodium pentobarbital, 80 mg/kg, i.p.) and perfused at physiological pressure with saline (0.9%) followed by 1% paraformaldehyde and 2% glutaraldehyde in 0.01 M phosphate-buffered saline (PBS, pH 7.4). Segments of the irradiated (right) and non-irradiated (left) femoral arteries were removed and kept in the same fixative buffer (pH 7.4) overnight at 4 °C prior to embedding. All arterial segments were embedded in paraffin, sectioned transversely (each section 3 μm thick) at 0.5 mm intervals, and the sections stained with hematoxylin and eosin. The intimal and medial areas were measured with the aid of a computer analysis system (FLOVEL VIDEOMICROMETER, model VM-30; OLYMPUS, Japan).

2.3. Thioglycollate-induced acute peritoneal inflammation

Twenty-nine intact mice were injected intraperitoneally with 1 ml of 3% thioglycollate broth (Difco, USA) or sterile pyrogen-free saline (sham). Just before this injection, the animals had received a subcutaneous injection of phytic acid (10 or 30 mg/kg), inositol (6 mg/kg), or vehicle. The mice were sacrificed 3 h after the peritoneal injection. Cells from the peritoneal cavity were collected by lavage with 9 ml of 0.01 M phosphate-buffered saline (PBS) containing 0.1% BSA, 0.54 mM EDTA, and 10 U/ml heparin to prevent clotting, and counted in a hemocytometer. The percentage of neutrophils was assessed by counting in cytospin preparations stained with Wright–Giemsa stain (Diff-Quik; Baxter, USA).

2.4. Cell proliferation and migration assays

2.4.1. Cell culture

Human coronary VSMCs were purchased from Sanko Junyaku Co. Ltd., Tokyo, Japan, and plated on 75 cm^2 flasks in a smooth muscle cell basal medium (SmBM; Sanko Junyaku Co. Ltd.) supplemented with 5% fetal bovine serum (FBS), 0.5 μg/ml human epidermal growth factor, 1 μg/ml basic fibroblast growth factor, 5 mg/ml insulin, 50 mg/ml gentamicin, and 50 mg/ml amphotericin-B under a humidified atmosphere of 5% CO_2 in air. The culture medium was changed every 2 days and a confluent VSMC monolayer was obtained after about 5 days. Cells were routinely used from the fourth to eighth passages.

2.4.2. Proliferation assay

The effects of phytic acid and inositol on cell proliferation were determined by a DNA synthesis assay method using 5-bromo-2′-deoxyuridine (BrdU) incorporation (Campana et al., 1988). Human coronary VSMCs were seeded into 96-well plates at a density of 2×10^4 cells/well in SmBM containing 5% FBS, and grown until confluence (4 days). Then, after the culture medium had been aspirated the cells were washed with medium containing 0.1% bovine serum albumin (BSA) and made quiescent by incubation for 2 days in the same medium. The medium was then replaced by fresh serum-free medium containing platelet-derived growth factor (PDGF)-BB (20 ng/ml) plus phytic acid, inositol, or saline. After 18 h, BrdU (10 μM) was added to each medium. After a 6-h incubation period, the incorporation of BrdU into DNA was measured using an enzyme immunoassay kit according to the recommendations of the manufacturer (BrdU Labeling and Detection Kit III; Boehringer Mannheim, Germany).

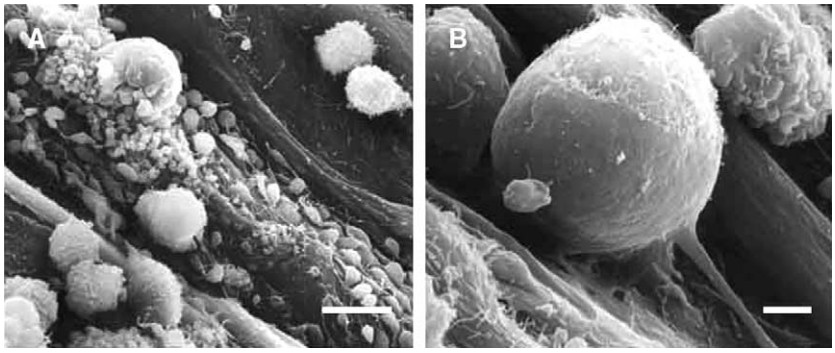

Fig. 1. Scanning electron microscopic observations of irradiated and non-irradiated segments of mouse femoral artery 3 days after endothelial injury the luminal surface is shown in each case. Bar = 10 μm (A) and 4.3 μm (B).

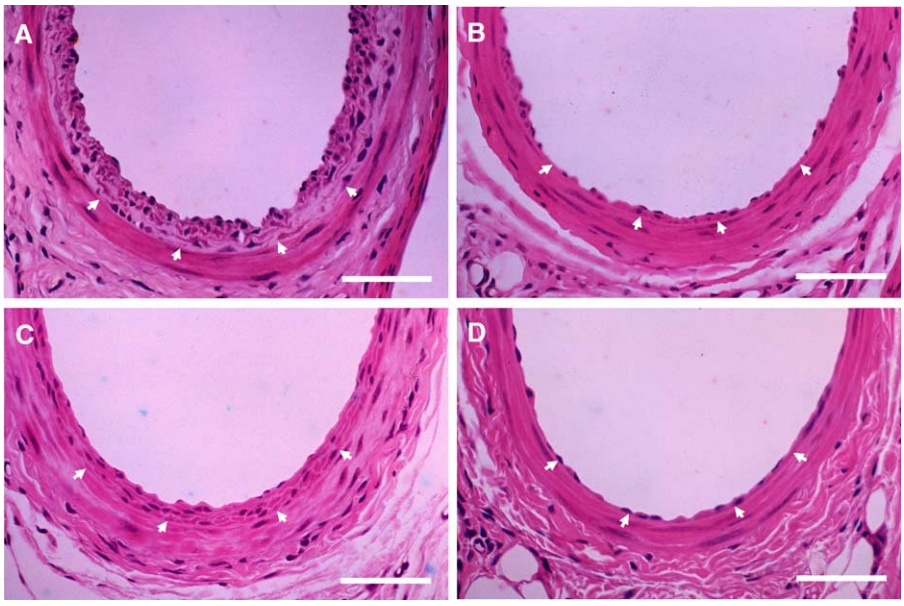

Fig. 2. Photomicrographs of transverse sections of mouse femoral artery 3 weeks after endothelial injury induced by a photochemical reaction. (A) Injured vessel from vehicle-treated control mouse for (B). (B) Injured vessel from mouse treated with anti-neutrophil antibody (i.p.) 3 days before the initiation of the photochemical reaction and then once a day for 10 days. (C) Injured vessel from vehicle-treated control mouse for (D). (D) Injured vessel from mouse treated with phytic acid (30 mg/kg, s.c.) 1 h after the initiation of the photochemical reaction and then twice a day for 7 days. Arrows indicate the internal elastic lamina. Bar = 50 μm.

2.4.3. Migration assay

Cells were removed from the flasks with 0.025% trypsin, centrifuged, and resuspended at 5×10^5 cells/ml in SmBM containing 0.1% BSA. The migration of human coronary VSMCs was assayed by the modified Boyden chamber method using a microchemotaxis chamber and polycarbonate membrane filter with pores of 8 μm diameter (Nucleopore, Pleasanton, CA, USA). Phytic acid, or inositol was placed in the upper and lower chambers, and PDGF-BB (20 ng/ml) was placed in the lower chamber. A cell suspension was loaded into the upper chamber and incubated for 4 h at 37 °C in a humidified atmosphere with 5% CO_2. The filter was stained with Wright–Giemsa stain (Diff-Quik; Baxter, USA). The number of human coronary VSMCs that had migrated to the lower surface of the filter was determined in 4 fields (0.25 mm^2) using a light microscope.

2.5. Drugs

Phytic acid and D-*myo*-inositol (inositol) were purchased from Aldrich (St. Louis, USA) and Wako (Osaka, Japan), respectively. Phytic acid and inositol were dissolved in sterile pyrogen-free saline and adjusted to pH 7.4 with HCl and NaOH. Anti-neutrophil antibody preadsorbed onto erythrocytes was purchased from Accurate Chemical and Scientific Corp. (Westbury, USA), and diluted with sterile pyrogen-free saline at the time of use.

2.6. Statistical analysis

Results are expressed as mean ± S.E.M. All data were analyzed using one-way analysis of variance (ANOVA) followed by Dunnett's test or Student's t-test. Differences were considered to be significant if $P < 0.05$.

3. Results

3.1. Leukocyte adhesion to the vessel wall

Scanning electron microscopy performed 1 day after the injury showed a number of platelets adhering to the vessel

Table 1
Effect of anti-neutrophil antibody on intimal hyperplasia after endothelial injury in mouse femoral artery

Treatment	Injured artery			Contralateral artery
	Area ($\times 10^{-2}$ mm^2)			Area ($\times 10^{-2}$ mm^2)
	Intima	Media	*I*/*M* ratio	Media
Control	0.641 ± 0.111	1.035 ± 0.067	0.708 ± 0.172	0.982 ± 0.038
Anti-neutrophil	0.269 ± 0.084[a]	1.164 ± 0.050	0.229 ± 0.076[a]	0.936 ± 0.026

Data are expressed as mean ± S.E.M. [a]$P < 0.05$ indicates significant difference from control (Student's *t*-test, $n = 12$).

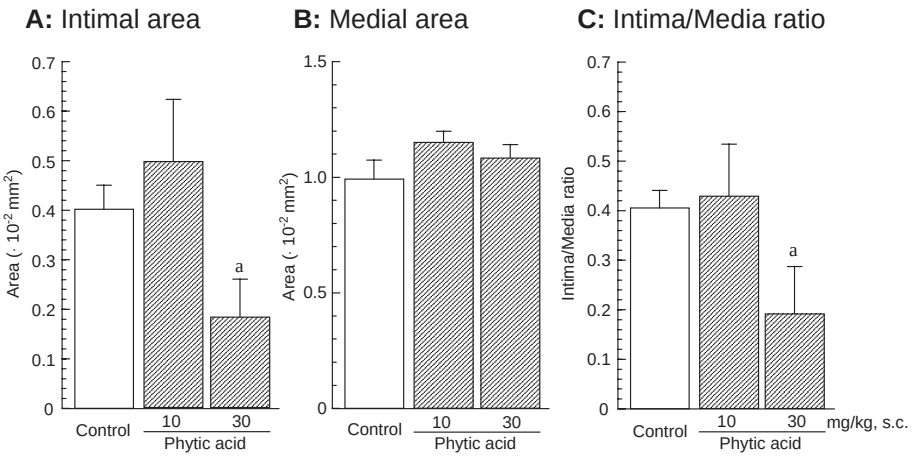

Fig. 3. Effect of phytic acid on intimal hyperplasia after endothelial injury in mouse femoral artery. Phytic acid or vehicle was administered subcutaneously 1 h after the endothelial injury and then twice a day for 7 days. Each column represents mean±S.E.M. (n=10 for each group). [a]$P<0.05$ indicates significant difference from vehicle-treated (control) group (one-way ANOVA followed by Dunnett's test).

wall at the injury site, while the wall of the contralateral (uninjured) artery was covered with a cobblestone-like layer of endothelial cells. Three days after the injury, a number of leukocytes could be seen adhering to platelets or to the subendothelial matrix at the site injury (Fig. 1). Scarcely any leukocyte adhesion was found on the wall of the injured vessel at 7 days after such injury.

3.2. Intimal hyperplasia

A total of 70 mice were used to determine the effects of neutropenia or phytic acid on the development of intimal hyperplasia after endothelial injury within the femoral artery. At 21 days after the such injury, neointimal formation was observed in the subendothelial layers in vehicle-treated animals (Fig. 2A and C). In the first series of experiments, mice that had received an endothelial injury were injected intraperitoneally with anti-neutrophil antibody, or an identical volume of vehicle (control), for 10 days. In a separate experiment, we confirmed that the neutrophil count in peripheral blood 3 days after such treatment with anti-neutrophil antibody is 90% less than in vehicle-treated animals, but that there is no change in the count of other leukocytes, erythrocytes, or platelets. In the present experiment, the intimal and medial areas were quantified using morphometric analysis at 21 days after the injury. Treatment with anti-neutrophil antibody (inducing neutropenia) produced a significant decrease in the intimal area and intima/media (I/M) ratio in the injured vessel (by comparison with the effects seen in the vehicle-treated control group) (Fig. 2 and Table 1). Such effects were not seen in the contralateral (uninjured) artery. Furthermore, the medial area in the ipsilateral artery was not affected by such treatment. In another series of experiments, treatment with phytic acid at 30 mg (33 μmol)/kg (s.c.), but not the

Table 2
Effects of phytic acid and inositol on neutrophil accumulation in the peritoneal cavity in mice with thioglycollate-induced acute peritoneal inflammation

	Dose (mg/kg, s.c.)	n	Neutrophils ($\times 10^6$ cells)
Sham		6	0.20±0.09[b]
Control		6	1.63±0.37
Phytic acid	10	6	0.99±0.24
	30	5	0.58±0.06[a]
Inositol	6	6	2.45±0.77

Data are expressed as the mean±S.E.M. [a]$P<0.05$, [b]$P<0.01$ vs. control (one-way ANOVA followed by Dunnett's test or Student's t-test).

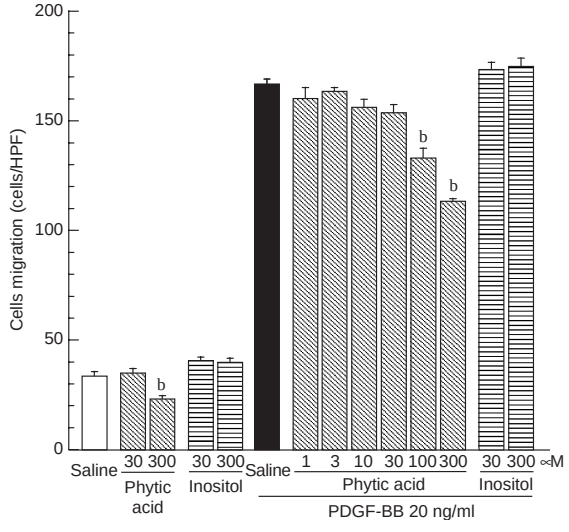

Fig. 4. Effects of phytic acid and inositol on migration of human coronary arterial smooth muscle cells induced by platelet-derived growth factor (PDGF)-BB. Phytic acid, inositol, or saline was applied 5 min before addition of PDGF-BB (20 ng/ml). Each column represents mean±S.E.M. (n=4). [b]$P<0.01$ indicates significant difference between PDGF-BB+phytic acid and PDGF-BB alone, and between phytic acid and saline (one-way ANOVA followed by Dunnett's test).

lower dose at 10 mg/kg (11 μmol)/kg (s.c.), produced a significant decrease in the intimal area and intima/media (*I*/*M*) ratio in the injured vessels by comparison with the effects seen in the vehicle-treated control group (Figs. 2C, D and 3). On the other hand, there were no significant differences in medial area of contralateral arteries between vehicle- and phytic acid-treated groups. Furthermore, there was no significant difference between inositol [6 mg (33 μmol)/kg, s.c.]-treated and control groups in terms of these measurements (data not shown).

3.3. Thioglycollate-induced acute peritoneal inflammation

In this model, treatment with phytic acid [10 mg (11 μmol)/kg or 30 mg (33 μmol)/kg, s.c.] reduced neutrophil accumulation in the peritoneal cavity 3 h after the thioglycollate injection, although the effect with the lower dose did not reach significance (Table 2). On the other hand, inositol [6 mg (33 μmol)/kg, s.c.] failed to inhibit such neutrophil accumulation (Table 2).

3.4. Migration and proliferation among human coronary vascular smooth muscle cells (VSMCs)

The effects of phytic acid and inositol on the migration (Fig. 4) and proliferation (Fig. 5) of human coronary VSMCs were examined. Phytic acid (1–300 μM), but not inositol (30 and 300 μM), concentration-dependently inhibited the migration induced by PDGF-BB (20 ng/ml) in these cells, the effect being significant at concentrations of 100 and 300 μM. Phytic acid (10–300 μM), but not inositol (100 and 300 μM), inhibited the PDGF-BB-induced proliferation of these cells in a concentration-dependent manner, this effect, too, being significant at concentrations of 100 and 300 μM.

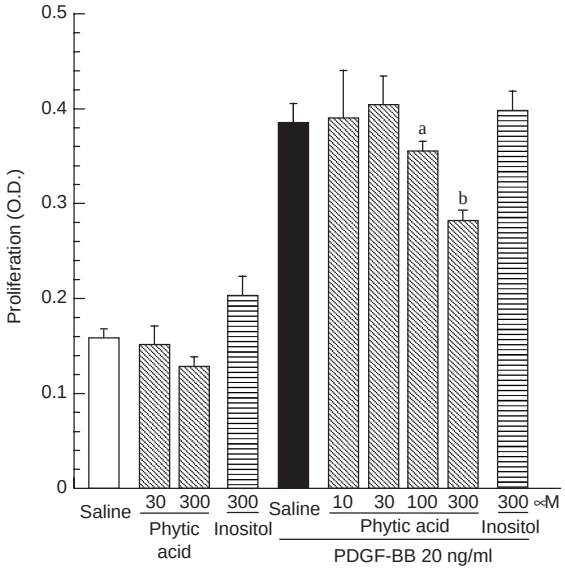

Fig. 5. Effects of phytic acid and inositol on proliferation of human coronary arterial smooth muscle cells induced by platelet-derived growth factor (PDGF)-BB. Phytic acid, inositol, or saline was applied 5 min before addition of PDGF-BB (20 ng/ml). Each column represents mean±S.E.M. (n=6). $^{a}P<0.05$, $^{b}p<0.01$ indicate significant differences between PDGF-BB+phytic acid and PDGF-BB alone and between phytic acid and saline (one-way ANOVA followed by Dunnett's test).

4. Discussion

In this study, we examined the participation of neutrophils and effect of phytic acid which have some properties such as inhibition of L- and P-selectin to sialyl Lewis^{X} and anti-oxidant in the development of intimal hyperplasia. To do this, we used a model of intimal hyperplasia developed in our laboratory which, unlike the balloon injury model, does not involve any mechanical stimulation of the media (Hirata et al., 1994; Kikuchi et al., 1998). In the present model, peroxidative damage to the endothelial membrane—which is achieved via the photodynamic generation of singlet molecular oxygen from injected rose bengal following irradiation of the artery with green light (540 nm)—provides the initial stimulus for the adhesion of platelets and their subsequent activation and aggregation, resulting in thrombus formation (Takiguchi et al., 1992). Formation of an occlusive thrombus is followed by naturally occurring reperfusion within 24 h in this model, and intimal hyperplasia is observed 7 days after the injury, reaching a plateau within 21 days (Kikuchi et al., 1998). In this study, 2 separate experiments as shown in Fig. 3 and Table 1 were carried out, and the degrees of intimal hyperplasia between 2 separate experiments showed differences. Although the reason for these differences is not clear, it may be derived from the subtle differences of experimental condition such as degree of endothelial injury, body weight, age and so on.

In this study, scanning electron microscopy revealed a large number of leukocytes adhering to platelets and to the subendothelial matrix in the vessel wall at the injury site 3 days after the infliction of the injury. These leukocytes were predominantly neutrophils from morphological observation in histological specimens (Shimazawa et al., unpublished data). Roque et al. (2000) also reported that the early accumulated leukocytes at the site of injury were predominantly neutrophils using femoral artery injured model in mice. On the other hand, this was not seen in the intact areas covered with a cobblestone-like layer of endothelial cells at sites outside the injured area or in the contralateral (uninjured) artery. These findings suggest that leukocyte, particularly neutrophils accumulation and activation at the site of an injury participate in the pathogenesis of intimal hyperplasia and restenosis.

In the present study, a depletion of circulating neutrophils in the peripheral blood induced by administration of anti-murine neutrophil antibody prior to the infliction of the endothelial injury was associated with a significant inhibition of the increase in the intimal area and intima/media (*I*/*M*) ratio that occurred in the controls following endothelial

injury. These results strongly suggest that neutrophil activation and accumulation at the site of an injury are responsible for the initiation and/or development of intimal hyperplasia. Bednar et al. (1985) reported that neutropenia (to 21±2% of control levels), induced using specific sheep anti-dog neutrophil antiserum, had minimal effects on platelet aggregation ex vivo, but significantly reduced platelet accumulation in the ischemic myocardium following a 5-h reperfusion, and also abolished the transmural distribution of platelets. They considered that interactions between platelets and neutrophils at the site of tissue damage may influence the processes resulting from a myocardial ischemic injury. In this study, we did not use a control antibody as vehicle-treated control group but identical volume of saline. Therefore, we could not exclude any influence of antibody itself in the thrombus formation, sensitivity of blood cells. However, in a separate experiment we confirmed that the neutrophil count in peripheral blood 3 days after such treatment with anti-neutrophil antibody is 90% less than in vehicle-treated animals, but that there is no change in the count of other leukocytes, erythrocytes, or platelets. Furthermore, there was no significant difference between neutropenia and control groups in terms of the time required to achieve thrombotic occlusion in the femoral artery following a photochemical reaction in vivo, suggesting similar degrees of platelet aggregation and endothelial damage. This result suggests that neutrophils have little effect on the thrombogenesis and response to endothelial injury that occurs in the mouse femoral artery in the acute phase. Accordingly, our result showing a reduced intimal hyperplasia in mice with neutropenia may reflect an attenuated neutrophil contribution in the phase following the reperfusion of the injured artery.

In this study, phytic acid—which inhibits the binding of L- (IC_{50}: 2.1±1.5 μM) and P-selectin-Ig-fusion protein (IC_{50}: 160±40 μM) to immobilized bovine serum albumin-sialyl Lewis^X (SLe^X) (Cecconi et al., 1994)—reduced the development of intimal hyperplasia in our mouse model. On the other hand, administration of inositol, which does not bind to L-, P-, or E-selectin, had little or no effect on the injury-induced increases in intimal area and I/M ratio. At the same doses, phytic acid, but not inositol, also reduced neutrophil accumulation (73% inhibition at 30 mg/kg, s.c.) in the peritoneal cavity of mice after thioglycollate injection. Furthermore, it has been reported that intimal hyperplasia can be reduced by blocking P- and E-selectin using an SLe^X analogue (Barrom et al., 1996), and that it is reduced in P-selectin knockout mice (Kumar et al., 1997). Collectively, these findings suggest that neutrophil adhesion mediated by L- and/or P-selectin and the subsequent activation of the neutrophils may lead to the development of intimal hyperplasia.

In the present study, we examined the effects of the neutropenia induced by an anti-murine neutrophil antibody and the effects of phytic acid—which possesses the ability, via its blockade of L- and P-selectin-binding to selectin ligands to inhibit leukocyte rolling during an inflammatory response—to try to clarify the participation of neutrophils and their adhesion molecules in the development of intimal hyperplasia. As might have been expected, neutropenia and treatment with phytic acid each separately reduced the development of intimal hyperplasia in our model. However, phytic acid was found directly to inhibit the migration and proliferation induced by PDGF-BB in cultured human coronary smooth muscle cells, although these effects were observed only at high concentrations (100 μM or more). Therefore, its suppression of intimal hyperplasia could derive from its anti-migration and/or anti-proliferation effects. However, phytic acid showed little effect on medial area at the contralateral arteries as compared with that of control group. Regarding another possible mechanism, it is known that phytic acid is able to inhibit the generation of hydrogen free radicals and the subsequent lipid peroxidation (Graf et al., 1987). To judge from the literature, one possible explanation for our results could be a protective effect of phytic acid against lipid peroxidation in the vascular smooth muscle cells following hydrogen radical generation from neutrophils or injured endothelial cells at the site of the injury (Rao et al., 1991). In view of this possibility, further studies will be necessary to clarify the details of the mechanisms involved.

In conclusion, the present study has demonstrated that both neutropenia and treatment with phytic acid each reduce the development of intimal hyperplasia in the mouse femoral artery. Accordingly, it is suggested that neutrophil accumulation on the injured vessels may contribute to the initiation and development of intimal hyperplasia, and its inhibitory effect of phytic acid might be mediated by L- and/or P-selectin at least in part. However, before reaching a definite conclusion it may be necessary to extend the investigation using selective agents with antagonistic properties against L-, P-, or E-selectin, when such agents are available. The elucidation of the mechanisms underlying the linkage between the inflammatory responses mediated by neutrophil adhesion/infiltration and the development of intimal hyperplasia may in time lead to novel approaches to the prevention of restenosis.

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