Photochemically Induced Endothelial Injury in the Mouse as a Screening Model for Inhibitors of Vascular Intimal Thickening

Shinji Kikuchi, Kazuo Umemura, Kazunao Kondo, Abby R. Saniabadi, Mitsuyoshi Nakshima

Abstract—We have established a mouse model of intimal thickening and assessed its suitability for experimental studies of intimal thickening. Neointimal formation was observed after endothelial injury by photochemical reaction between transluminal green light and systemically administered rose Bengal, which represents a nonmechanical approach to vessel wall denudation. Intimal thickening began 7 days after endothelial injury, reached a maximum after 21 days, and then remained unchanged for as long as 42 days. Furthermore, as a consequence of neointimal proliferation, the luminal area gradually decreased. The cells in the neointimal layer were identified as smooth muscle cells by immunohistochemical staining with an α-actin–specific antibody. Extracellular matrix deposition in the neointima was markedly increased beyond 14 days after injury. Smooth muscle cell proliferation, as measured by pulse labeling of 5-bromo-2′-deoxyuridine, was identified initially in the media 2 days after vessel wall denudation, with the proliferative activity’s shifting almost exclusively to the neointima within 7 days. Endothelial regeneration, as indicated by Evans blue staining, was complete within 21 days after injury. To assess the suitability of this model for experimental studies on intimal thickening, the effect of tranilast, an antiallergy drug with a broad spectrum of pharmacological actions on intimal thickening, was investigated. Tranilast (100 mg · kg⁻¹ · d⁻¹ PO) significantly (P<0.05) reduced smooth muscle cell proliferation in the neointima and media 7 days after injury and neointimal formation 21 days after injury in treated mice compared with vehicle-treated mice. This simple experimental mouse model is suitable for studying factors promoting or inhibiting intimal thickening after endothelial injury and for developing therapeutic strategies against intimal thickening. (Arterioscler Thromb Vasc Biol. 1998;18:1069-1078.)

Key Words: mouse • endothelial injury • intimal thickening • smooth muscle cell proliferation • tranilast

It is believed that intimal thickening occurs with the progression of atherosclerosis and the development of restenosis after coronary angioplasty. To date, several experimental approaches to induce intimal thickening have been reported. These include flexible wire, balloon catheter, air drying, and laser injury. Although such injuries do lead to neointimal formation in arteries by removing the endothelium, which can then be used for the study of atherosclerosis and restenosis after angioplasty, they all involve procedures that are relatively complicated and are difficult to control with respect to the extent of vascular injury. In light of these situations, establishment of a simple and reproducible animal model of intimal thickening that occurs in response to endothelial injury would be very desirable to assist in gaining insight into the pathophysiology of neointimal formation after vascular injury and in screening potential therapeutic agents.

We have previously reported a simple and reproducible thrombosis model that makes use of the photochemical reaction between transluminal green light (540 nm) and systemically administered rose Bengal. This model represents a nonmechanical and noninvasive method of achieving vessel wall denudation. In this study, we adapted this photochemical model of thrombosis to induce intimal thickening in the mouse femoral artery. The mouse has considerable advantages over other animals such as the rat, rabbit, and guinea pig. First, the quantity of agents, antibodies, etc required to investigate stimulation or prevention of intimal thickening can be small. Second, transgenic or gene-knockout mice are now available for identifying factors responsible for the development of intimal thickening. Third, mice are readily available, easy to handle, and inexpensive to purchase and maintain.

Tranilast, (3,4-dimethoxycinnamoyl)anthranilic acid, has been in clinical use in Japan as an effective antiallergy and antikeloid drug. Recently, it has been reported that this drug markedly inhibited the proliferation and migration of SMCs in vitro. Furthermore, a double-blind, large-scale, multicenter phase III trial of tranilast at a dose of 600 mg/d for 3 months reduced the rate of post–percutaneous transluminal coronary angioplasty restenosis compared with placebo.

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The principal aim of the current study was to establish a mouse model of intimal thickening and assess its suitability for evaluating pharmacological preparations that may suppress intimal thickening. Endothelial injury was caused by photochemical reaction between transluminal green light and intravenously administered rose Bengal, which represents a nonmechanical approach of producing arterial denudation.

Methods

Vessel Wall Injury

Vascular injury in the mouse femoral artery was photochemically induced as described previously. In brief, a total of 176 male ICR mice (5 weeks old, 25 to 30 g) were anesthetized with sodium pentobarbital (80 mg/kg IP), and a cannula was inserted into the jugular vein for rose Bengal injection. The right femoral artery was carefully exposed, and the probe of a laser monitor (model ALF 2100, Advance Co) was attached to the branch point of the deep jugular vein for photochemical treatment. The irradiation was directed with use of a heat-absorbing filter prevents excessive generation of heat that can damage biological tissues. The irradiation was performed 13 minutes after baseline blood flow had completely stopped. Thirty minutes later the femoral artery segments were removed and fixed with 1% paraformaldehyde and 2% glutaraldehyde in 0.1 mol/L PBS, pH 7.4, as described by Saniabadi et al., after most of the blood had been drained from the specimen. The media was defined as the region between the internal and external elastic laminae. The cross-sectional areas of the neointima and media (1 section per animal) and the number of intimal and medial SMCs (5 sections per animal) were measured by using a computerized apparatus (Videoplan). Measurements were made in blinded manner.

BrdU Labeling

BrdU labeling was performed to investigate the number of SMCs undergoing DNA synthesis. Mice were injected intraperitoneally with BrdU (30 mg/kg) and 5-fluoro-2-deoxyuridine (3 mg/kg, both from Sigma) at 18, 6, and 1 hour before they were euthanized. Arterial segments labeled with BrdU were excised at 2, 7, 14, and 21 days after endothelial damage. After fixation with methyl Carnoy’s fixative (60% methanol, 30% chloroform, and 10% glacial acetic acid), embedding, consecutive sectioning, and immunohistological staining, the number of proliferating cells was determined in both medial and intimal areas of arterial sections. Measurements were made in a blinded manner on 5 sections from each injured artery. The medial and intimal proliferation indices were taken as the percentage of the total number of cells in the media and intima, respectively, that were BrdU-positive.

Immunocytochemistry

For immunohistochemical examination, all tissue samples were fixed with methyl Carnoy’s fixative, embedded in paraffin, and sectioned. Immunohistochemical staining of the deparaffinized sections was performed by using the labeled streptavidin biotin method at SAB kit, (1:100, BSC) for 2 hours at room temperature. Biotinylated anti-rat antibodies (1:600, Dako) were applied for 30 minutes at room temperature as a secondary antibody. After the sections had been rinsed in 0.15 mol/L NaCl containing 0.05 mol/L Tris HCl buffer, pH 7.6, peroxidase-labeled streptavidin was added to the slide. Antibody visualization was established after a 5-minute exposure to 0.05% 3,3′-diaminobenzidine 4HCl in Tris-buffered saline with 0.03% H2O2. To enable identification of SMCs, sections were incubated with an anti-human smooth muscle actin antibody, 1A4 (Enhanced Polymer One-Step Staining system, Dako) for 12 hours at 4°C, followed by color development with 3-amin-9-ethylcarbazole. All sections were counterstained with Mayer’s hematoxylin.

Scanning Electron Microscopy

For scanning electron microscopy, animals were perfusion fixed with PBS containing 0.7% paraformaldehyde and 2% glutaraldehyde, pH 7.4, as described by Sanjabi et al., after most of the blood had been washed out with saline. The irradiated femoral artery segment was then carefully removed and placed in the same fixative for 2 hours at 4°C. After the fixative had been replaced with PBS, the specimens were dehydrated in graded ethanol and dried in a liquid CO2 freeze-drying device (JFD-300, JEOL). Each segment was etched by the ion-beam bombardment method to allow visual inspection and was processed further for scanning electron microscopy.

Evaluation of Reendothelialization

Reendothelialization was assessed by staining the deendothelialized areas of the femoral artery with Evans blue dye as previously described. In brief, a 50-μL portion of a 5% dye solution was injected into the tail vein with the use of a 27-gauge needle 10 minutes before the mice were euthanized. After the mice had been perfused fixed as described above, the injured segment was dissected while being viewed under a microscope and then photographed. The length of the blue-stained, denuded area was measured with a computerized apparatus (Videoplan).

Histological and Morphological Procedures

Fixed femoral artery segments were embedded in paraffin and cut consecutively into 5-μm-thick sections. Sections were taken for study at 500-μm intervals. The sections were stained with hematoxylin and eosin or Masson’s trichrome reagent. For morphometric analysis, hematoxylin and eosin–stained sections were used. The neointima was defined as the region between the lumen and the internal elastic lamina. The media was defined as the region between the internal and external elastic laminae. The cross-sectional areas of the neointima and media (1 section per animal) and the number of intimal and medial SMCs (5 sections per animal) were measured by using a computerized apparatus (Videoplan). Measurements were made in blinded manner.
Figure 1. Scanning electron photomicrographs of mouse injured femoral artery surface. A and B, Immediately after photochemically induced endothelial injury; C, D, E, and F, on days 1, 3, 7, and 21, respectively. In A and B, thrombus is composed of distorted red blood cells and leukocytes trapped in a large amount of fibrin, together with numerous aggregated platelets. B is a higher magnification of A. C, Spontaneous thrombolysis within 24 hours of thrombus occlusion of the vessel. Numerous activated platelets are still evident adherent to injured surface. D, Platelets and leukocytes have formed a monolayer on injured surface. E, No leukocytes are visible on injured surface, but few platelets remain. F, No blood cells on arterial injured surface.
Drug Administration

Tranilast, a gift from Kissei Pharmaceuticals Japan, was suspended in 0.5% carboxymethylcellulose and administered orally once a day at 100 mg/kg from 2 hours after endothelial injury. Control animals received an equal volume of 0.5% carboxymethylcellulose. The effect of tranilast on BrdU incorporation and intimal thickening was evaluated at 7 and 21 days, respectively. The effect of tranilast on endothelial regeneration was evaluated 7 and 21 days after injury.

Figure 2. Light photomicrographs of serial histological cross sections from mouse femoral artery 21 days after photochemical injury. A-E and F are cross sections from injured and uninjured vessels, respectively. Internal elastic lamina is indicated by arrowheads in A-F. Hematoxylin and eosin stain, bar=50 μm.
TABLE 1. Topographic Analysis of Neointimal Thickening 21 Days After Photochemically Induced Endothelial Injury

<table>
<thead>
<tr>
<th>Intima-Media Ratio of Topographic Positions in Injured and Uninjured Segments</th>
<th>Injured</th>
<th>Uninjured</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.130±0.022*</td>
<td>0.358±0.046*</td>
</tr>
<tr>
<td>B</td>
<td>0.358±0.046*</td>
<td>0.668±0.131</td>
</tr>
<tr>
<td>C</td>
<td>0.668±0.131</td>
<td>0.356±0.038*</td>
</tr>
<tr>
<td>D</td>
<td>0.356±0.038*</td>
<td>0.174±0.040*</td>
</tr>
<tr>
<td>E</td>
<td>0.174±0.040*</td>
<td>0.000±0.000*</td>
</tr>
<tr>
<td>F</td>
<td>0.000±0.000*</td>
<td></td>
</tr>
</tbody>
</table>

Data are presented as mean±SEM derived from 12 animals. A–E in the table indicate the schematically represented artery in Figure 2.

Statistics

Results are presented as mean±SEM. Differences between groups were analyzed with Dunnett’s multiple-comparison test. For differences between two groups, two-tailed, unpaired, Student’s t test was used. A value of P<0.05 was considered significant.

Results

Photochemically Induced Endothelial Injury

The mouse femoral artery became thrombocytically occluded (cessation of blood flow) ≈13 minutes after systemic rose Bengal injection and green light irradiation. Twenty-four hours later, spontaneous reflow, which was observed in all mice, had recovered to ≈86% of the baseline level.

Scanning Electron Microscopy

The scanning electron photomicrograph in Figure 1A shows a full view of the femoral artery occluded by a thrombus. Figure 1B is a view at higher magnification of part of Figure 1A. In this model, the thrombus is composed of distorted red blood cells and leukocytes trapped in a fibrin mesh, together with a large number of aggregated platelets (Figure 1B). One day after the thrombotic occlusion, spontaneous thrombolysis had occurred, but numerous activated platelets were still seen adherent to the injured arterial wall (Figure 1C). After 3 days, in addition to platelets, leukocytes were also present and had formed a monolayer on the injured vessel wall (Figure 1D).

Seven days after the injury, no leukocytes were seen in the damaged area, but a few platelets were still seen adherent to the exposed subendothelial matrix (Figure 1E). Twenty-one days after the operation, no blood cells were present on the luminal surface (Figure 1F).

Light Microscopy

Twenty-one days after vessel denudation, an extensive neointima had formed in the subendothelial layers throughout the injured arterial segment. Figure 2 shows a typical pattern of intimal thickening in this study. A neointima was formed from borders between uninjured and injured sites (positions A and E in Figure 2) into the injured center (position C in Figure 2). On the other hand, neointimal formation was not observed in the normal site (position F in Figure 2). This process was quantified by measuring the intima-media ratio at almost equally spaced positions across the injured segment in Figure 2 (Table 1). Position C in Figure 2 had a significantly thickened neointima compared with neighboring positions (Table 1, P<0.001). Therefore, in all cases, the section showing the greatest intima-media ratio was selected for planimetry. Within 24 hours after endothelial injury, the number of medial SMCs had decreased by ≈28% compared with uninjured controls, and the loss of medial SMCs was apparent in some sections after endothelial denudation. Statistical analysis, however, revealed no significant decrease in medial cell number between any group. Seven days after injury, SMCs that had migrated from the media were present in the neointima and it became gradually thicker, coincident with its repopulation by cells. Neointimal formation reached a maximum 21 days after endothelial injury and remained unchanged for as long as 42 days after the injury. Within 2 days after endothelial injury, the luminal area decreased, reflecting vasoconstriction due to the disappearance of endothelium-dependent vasodilatation, but this initial decrease in luminal area was reversed at 7 days after injury. Thereafter,

TABLE 2. Time Course of Changes in Intimal and Medial Cells; Luminal, Intimal, and Medial Cross-sectional Areas; and Intima-Media Ratio After Photochemically Induced Endothelial Injury

<table>
<thead>
<tr>
<th>Time After Injury</th>
<th>Control</th>
<th>30 Minutes</th>
<th>24 Hours</th>
<th>2 Days</th>
<th>7 Days</th>
<th>14 Days</th>
<th>21 Days</th>
<th>42 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intimal cells</td>
<td>0±0</td>
<td>0±0</td>
<td>0±0</td>
<td>0±0</td>
<td>31±5†</td>
<td>70±8‡</td>
<td>72±8‡</td>
<td>83±15‡</td>
</tr>
<tr>
<td>Medial cells</td>
<td>46±3</td>
<td>32±4</td>
<td>34±2</td>
<td>38±5</td>
<td>44±3</td>
<td>45±3</td>
<td>42±2</td>
<td>41±3</td>
</tr>
<tr>
<td>Area, ×0.01 mm²</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Luminal</td>
<td>1.879±0.226</td>
<td>1.578±0.462</td>
<td>1.519±0.427</td>
<td>0.617±0.093†</td>
<td>1.653±0.638</td>
<td>1.518±0.258</td>
<td>1.204±0.208</td>
<td>1.267±0.166</td>
</tr>
<tr>
<td>Intimal</td>
<td>0±0</td>
<td>0±0</td>
<td>0±0</td>
<td>0±0</td>
<td>0.239±0.079*</td>
<td>0.301±0.049†</td>
<td>0.609±0.079‡</td>
<td>0.662±0.094‡</td>
</tr>
<tr>
<td>Medial</td>
<td>0.941±0.047</td>
<td>1.059±0.136</td>
<td>0.859±0.058</td>
<td>0.935±0.044</td>
<td>1.245±0.079</td>
<td>1.216±0.149</td>
<td>1.014±0.073</td>
<td>1.136±0.159</td>
</tr>
<tr>
<td>Intima-media ratio</td>
<td>0±0</td>
<td>0±0</td>
<td>0±0</td>
<td>0±0</td>
<td>0.180±0.046</td>
<td>0.276±0.058‡</td>
<td>0.622±0.086‡</td>
<td>0.661±0.121‡</td>
</tr>
<tr>
<td>n</td>
<td>10</td>
<td>10</td>
<td>8</td>
<td>10</td>
<td>9</td>
<td>10</td>
<td>10</td>
<td>8</td>
</tr>
</tbody>
</table>

Data are presented as mean±SEM.

*P<0.05, †P<0.01, and ‡P<0.001 vs control value.
with neointimal proliferation, the luminal area of the vessel decreased, but the medial area remained unchanged throughout the observation period (Table 2).

Figure 3 (A, B, and C) shows a progressive increase in the area positive for smooth muscle actin. There were only a few α-actin-positive areas in the neointima at 7 days after the injury (Figure 3A). By day 21, however, almost the entire neointima was positive for α-actin (Figure 3B and 3C). ECM deposition surrounding SMCs in the neointima was confirmed by Masson’s trichrome staining. There was only a small amount of ECM in the neointima at 7 days after injury (Figure 3D). Beyond 14 days after injury, however, a significant amount of ECM was present in the neointima (Figure 3E and 3F). This finding suggests that the neointima that had formed after the vascular injury was composed mainly of SMCs and ECM.

**Endothelial Regrowth**

Immediately after vessel wall injury, ECs from the photoirradiated segment were completely absent, and blue staining...
was revealed (Figure 4B); no blue-stained area was observed in the uninjured vessel (Figure 4A). ECs originating from the uninjured borders grew into the injured center, and the reendothelialization was complete within 21 days after injury (Figure 4C, 4D, and 4E; Table 3).

**Cell Proliferation**

Arterial cell proliferation as indicated by positive BrdU staining occurred as shown in Table 4. SMC proliferation in the medial layer had started 2 days after vessel wall injury. The maximum level detected was at 2 days, which gradually decreased to baseline level by day 21. Positive staining in intimal cells was first observed 7 days after the endothelial injury and was maximum at this point, the ratio of BrdU labeling thereafter decreasing in a time-dependent manner.

**Effect of Tranilast on BrdU Incorporation, Intimal Thickening, and Endothelial Regeneration**

Mice were assigned to 2 groups after the thrombotic occlusion of the injured femoral artery: the vehicle group and the tranilast group. The mouse femoral artery was occluded 13.0±1.8 and 9.8±1.2 minutes in the control and tranilast groups, respectively. There was no significant difference in occlusion time between the two groups.

The effect of tranilast on SMC proliferation as evidenced by BrdU labeling in the neointima and media of the femoral artery is presented in Figure 5. Mice were given tranilast (100 mg/kg) orally for 7 days, starting 2 hours after the vessel injury; the vessel segments were taken 7 days after the initiation of the injury. Tranilast significantly reduced the number of proliferating SMCs of the neointima compared with vehicle-treated control mice (15.0±2.5% versus 38.5±7.1%, P<0.05). Likewise, a significant reduction in medial SMC proliferation was seen between tranilast- and vehicle-treated mice (2.4±1.2% versus 8.1±2.0%, P<0.05).

The effect of tranilast on intimal thickening is presented in Table 5. Mice were given tranilast orally for 21 days, starting 2 hours after the vessel injury; the vessel segments were taken 21 days after the initiation of the injury. Administration of tranilast significantly reduced the cross-sectional area of the neointima compared with vehicle-treated control mice (0.317±0.041 versus 0.587±0.059 mm², P<0.01). The area of the media in the tranilast-treated mice was almost the same as in the control mice. The values for the intima-media ratio were markedly less in the tranilast-treated mice compared with vehicle-treated control mice (0.338±0.041 versus 0.644±0.083, P<0.01). The luminal sizes were significantly increased in the tranilast-treated mice compared with the vehicle-treated mice (2.849±0.143 versus 1.360±0.270×0.01 mm², P<0.001).

The effect of tranilast on endothelial regeneration was investigated at 7 and 21 days after vessel denudation by using Evans blue staining. Tranilast did not affect endothelial regeneration after endothelial injury (Figure 6).

**Discussion**

In the current study, we adapted a photochemical model of thrombosis to induce intimal thickening due to neointimal formation in the mouse femoral artery and investigated the

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**TABLE 3. Time Course of Changes in Endothelial Regrowth (mm) After Photochemical Vessel Wall Injury in the Mouse Femoral Artery**

<table>
<thead>
<tr>
<th>Time After Injury</th>
<th>Uninjured</th>
<th>2 Hours</th>
<th>2 Days</th>
<th>7 Days</th>
<th>21 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.000±0.000</td>
<td>3.603±0.132</td>
<td>3.444±0.015</td>
<td>1.505±0.412</td>
<td>0.000±0.000</td>
</tr>
</tbody>
</table>

Data are presented as mean±SEM from 4–6 animals.

**TABLE 4. Time Course of Proliferation of Intimal and Medial SMCs After Photochemically Induced Endothelial Injury**

<table>
<thead>
<tr>
<th>Time After Injury, BrdU-Positive Cells ×100/All Cells (%)</th>
<th>Control</th>
<th>2 Days</th>
<th>7 Days</th>
<th>14 Days</th>
<th>21 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intima</td>
<td>0±0</td>
<td>0±0</td>
<td>38.5±7.1</td>
<td>12.2±4.8</td>
<td>5.3±3.4</td>
</tr>
<tr>
<td>Media</td>
<td>0±0</td>
<td>17.1±3.8</td>
<td>8.1±2.0</td>
<td>3.7±2.5</td>
<td>2.3±2.3</td>
</tr>
</tbody>
</table>

Data are presented as mean±SEM.

---

**Figure 4.** Photographs showing Evans blue staining of mouse femoral arteries after endothelial injury. A, Control (no blue staining in uninjured artery); B, immediately after injury; C, D, and E are 2, 7, and 21 days after injury, respectively. Evans blue dye penetrated the injured segment immediately after endothelial injury. Blue-stained areas gradually decreased thereafter and were absent by 21 days after injury, at which time endothelial regeneration was complete. Bar=5 mm.
time course of morphological and proliferative changes after the endothelial injury. This model involves a photochemical reaction between transluminal green light and systemically administered rose Bengal, which causes endothelial injury followed by platelet adhesion, aggregation, and formation of a platelet- and fibrin-rich thrombus at the site of the reaction and represents a nonmechanical approach to cause endothelial injury and vessel denudation. Scanning electron photomicrographs revealed that immediately after the photochemical reaction, an occlusive thrombus formed and was composed of adherent and aggregated platelets, leukocytes, and a large amount of fibrin. Within 24 hours of thrombotic occlusion, reflow was observed due to spontaneous thrombolysis; however, there were still large numbers of platelets and leukocytes adherent to the injured arterial surface.

Neointimal formation after vascular injury reflects migration of SMCs from the media to the intima within the arterial wall, proliferation of SMCs in the intima, and excessive production of ECM by SMCs.13 A number of studies have reported that certain growth factors, including platelet-derived growth factor,14 basic fibroblast growth factor,15 interleukin-1β,13 and thrombin16 derived from microthrombi, activated platelets, and leukocytes or the injured ECs themselves, may interact with the SMCs in an autocrine or paracrine manner to promote SMC proliferation and migration, thus contributing to the neointimal formation.

In human restenosis, an occlusive thrombus forms in 3% to 5% of patients within the first few hours after angioplasty, and deposition of nonadherent thrombi at the restenosing lesion has been reported in 40% of patients.17 Hence, the initial response in our model, despite involving no mechanical damage to the media, appears to be similar to the phenomenon seen after balloon angioplasty in humans.

In this model of photochemically induced endothelial injury in the mouse femoral artery, we produced intimal thickening in 100% of the femoral arteries investigated, with zero morbidity. The neointima in the femoral arteries first showed thickening 7 days after arterial injury, with the effect reaching a plateau that could be observed for as long as 42 days after the injury. As a consequence of neointimal formation, the luminal area decreased, similar to the intimal thickening that has been observed after balloon catheter injury in the rat carotid artery.2

Immunostaining for α-actin as an SMC marker indicated that cells in the neointima were almost entirely SMCs; in fact, there was a progressive increase in the α-actin-positive content. ECM accumulation was absent from the neointima 7 days after arterial injury. Beyond 14 days after injury, however, a significant amount of ECM was present surrounding the SMCs in the neointima. We found that the peak proliferative activity of SMCs occurred in the media at 2 days, and the neointima peak was observed later, at 7 days. It follows, therefore, that an increase in medial SMC replication was followed by migration of SMCs into the intima, ~7 days after the injury. The number of SMCs undergoing DNA synthesis gradually decreased between days 7 and 21. However, the intimal area increased during these 21 days, despite decreasing DNA synthesis, which may reflect proliferation of a large number of cells soon after vascular injury and a progressive increase in ECM synthesis from 14 to 21 days after injury.

TABLE 5. Effect of Tranilast on Luminal, Intimal, and Medial Cross-sectional Areas and Intima-Media Ratio in the Mouse Femoral Artery

<table>
<thead>
<tr>
<th></th>
<th>Luminal Area*</th>
<th>Intimal Area*</th>
<th>Medial Area*</th>
<th>I-M Ratio</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.360±0.270</td>
<td>0.587±0.059</td>
<td>0.960±0.062</td>
<td>0.644±0.083</td>
<td>10</td>
</tr>
<tr>
<td>Tranilast (100 mg/kg)</td>
<td>2.849±0.143‡</td>
<td>0.317±0.041†</td>
<td>0.958±0.072</td>
<td>0.338±0.041†</td>
<td>10</td>
</tr>
</tbody>
</table>

Tranilast (100 mg/kg) was given orally for 21 days, starting 2 hours after vessel wall injury. Vessels were harvested 21 days after the injury. I-M ratio indicates the intimal area-medial area ratio. Data are presented as mean±SEM.

*P<0.01 mm².
†P<0.01 and ‡P<0.001.
lumen size seen in this study. In the assessment of intimal
luminal surface was fully covered with new ECs.
vascular injury: the proliferation of SMCs and the increase
in lumen size observed 21 days after vascular injury. Ihara et al.20
after vascular injury. Furthermore, tranilast increased the
inhibitory effect of tranilast on intimal thickening is likely
corresponding to its inhibition of SMC proliferation and migration in vitro, respective-
ly (S.K. et al., unpublished observations, 1996). This indicates that tranilast did not influence vessel injury during the first 2 hours after photochemical reaction.
In a phase III clinical trial, tranilast at a dose of 600 mg/d
(200 mg TID) for 3 months reduced the rate of post–percu-
taneous transluminal coronary angioplasty restenosis.11 When tranilast (200 mg, single dose) was administered orally to patients with angina, the maximum plasma concentration of the drug was 31.7 µg/mL (Dr. Hideo Tamai, personal communication, 1997). On the basis of this result, we estimated the plasma concentration of tranilast achieved when the drug, at 200 mg, was administered 3 times a day for 7 days in humans by using a nonlinear least-squares regression computer program (Win Nonlin). The estimated plasma concentration of tranilast increased to 73.0 µg/mL, which is comparable to the 83.2 µg/mL in nonfasted mice reported in this study.

One of the characteristics of the mouse model of vascular injury is the possibility of using transgenic or gene-knockout mouse for identifying factors responsible for the development of intimal thickening. In this study, we used the ICR mouse strain for the induction of intimal thickening. However, the ICR mouse strain is not an inbred strain that is commonly used in transgenic and gene-knockout technology. Therefore, the usefulness of this model in commonly used inbred strains still needs to be tested.

In conclusion, the mouse model of intimal thickening described here is a simple and practical approach to study SMC replication, migration, and neointimal formation. It can serve as an in vivo screening model to evaluate drugs for antiproliferative activity.

References

Figure 6. Effect of tranilast on endothelial regeneration in mouse femoral artery measured at times indicated after photochemical injury. Data are mean±SEM for n=4–6 mice.


Photochemically Induced Endothelial Injury in the Mouse as a Screening Model for Inhibitors of Vascular Intimal Thickening

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