Tranilast Suppresses Vascular Chymase Expression and Neointima Formation in Balloon-Injured Dog Carotid Artery

Naotaka Shiota, MD, PhD; Hideki Okunishi, MD, PhD; Shinji Takai, PhD; Imao Mikoshiba, PhD; Hiroshi Sakonjo, PhD; Nobuo Shibata, PhD; Mizuo Miyazaki, MD, PhD

Background—Activation of vascular chymase plays a major role in myointimal hypertrophy after vascular injury by augmenting the production of angiotensin (ANG) II. Because chymase is synthesized mainly in mast cells, we assumed that the chymase-dependent ANG II formation could be downregulated by tranilast, a mast cell–stabilizing antiallergic agent. We have assessed inhibitory effects of tranilast on neointima formation after balloon injury in the carotid artery of dogs, which share a similar ANG II–forming chymase with humans, and further explored the pathophysiological significance of vascular chymase.

Methods and Results—Either tranilast (50 mg/kg BID) or vehicle was orally administered to beagles for 2 weeks before and 4 weeks after balloon injury. Four weeks after the injury, remarkable neointima was formed in the carotid arteries of vehicle-treated dogs. Chymase mRNA levels and chymaselike activity of vehicle-treated injured arteries were increased 10.2- and 4.8-fold, respectively, those of uninjured arteries. Angiotensin-converting enzyme (ACE) activity was slightly increased in the injured arteries, whereas ACE mRNA levels were not. Tranilast treatment completely prevented the increase in chymaselike activity, reduced the chymase mRNA levels by 43%, and decreased the carotid intima/media ratio by 63%. In vehicle-treated injured arteries, mast cell count in the adventitia showed a great increase, which was completely prevented by the tranilast treatment. Vascular ACE activity and mRNA levels were unaffected by tranilast.

Conclusions—Tranilast suppressed chymase gene expression, which was specifically activated in the injured arteries, and prevented neointima formation. Suppression of the chymase-dependent ANG II–forming pathway may contribute to the beneficial effects of tranilast. (Circulation. 1999;99:1084-1090.)

Key Words: angiotensin ■ leukocytes ■ restenosis ■ angiogenesis ■ polymerase chain reaction

Percutaneous transluminal coronary angioplasty (PTCA) is an established therapy for obstructive coronary artery diseases. However, its long-term efficacy is limited by frequent occurrence of restenosis.1 Migration and proliferation of smooth muscle cells (SMCs) with subsequent formation of neointima are considered important in late stenosis after PTCA.1 In this process, angiotensin (ANG) II supposedly plays a major role.2 Involvement of ANG II in the pathogenesis of restenosis was further confirmed by the facts that ACE inhibitors as well as ANG II receptor antagonists effectively prevented neointima formation in balloon-injured rats.3,4 On the basis of these experimental findings, ACE inhibitors were applied in clinical trials for preventing restenosis after PTCA. However, ACE inhibitors proved to be ineffective on human restenosis.5,6

Such species differences in the ACE inhibitor effects on neointima suggested the presence of alternative ANG II–forming pathways in humans. Previously we demonstrated that chymase contributed to ANG II formation in human, monkey, and dog vascular tissues.7-11 Chymases of these species cleave the Phe8-His9 bond of ANG I and produce ANG II efficiently.7-13 In contrast, rat chymase (rat mast cell protease I [RMCP-1]) hydrolyzes the Tyr4-Ile5 bond to yield inactive fragments.14 Therefore the vascular ANG II–forming system in primates and dogs is quite different from that in rats.9 Such species differences in the vascular ANG II–forming systems may explain the differential effects of ACE inhibitors on myointimal hypertrophy. To confirm our hypothesis, we established balloon-injured models of dogs that have the dual ANG II–forming systems like that in humans and compared the effects of an ACE inhibitor and an ANG II receptor antagonist. Balloon injury remarkably activated canine vascular chymase,10 and an ANG II receptor antagonist substantially prevented neointima formation, whereas an ACE inhibitor did so only modestly.15 These results indicate that chymase contributes to the pathogenesis of intimal hyperplasia by augmenting the local ANG II production to a greater extent than does ACE.

Chymase is synthesized mainly in connective tissue–type mast cells and secreted into the interstitium. Some antiallergic...
drugs are capable of stabilizing mast cell functions. Therefore the chymase-dependent ANG II formation may be attenuated by such agents that suppress mast cell activation. Tranilast, N-(3,4-dimethoxyphenyl)anthranilic acid, is an antiallergic drug that has clinical indications such as bronchial asthma, allergic rhinitis, atop dermatitis, and keloid and hypertrophic scar. Thus we investigated in dogs whether tranilast inhibits neointima formation and whether vascular chymase is involved in the pathogenesis of neointimal hyper trophy after balloon injury.

**Methods**

**Balloon Catheterization of Carotid Arteries**

Twelve male beagles weighing 9 to 12 kg (Nihon Nosan, Yokohama, Japan) underwent balloon catheterization as described previously with modification. Under pentobarbital anesthesia, the anterior thyroid arteries of both right and left sides were exposed. Through the right side artery, a balloon catheter (3F, VERMED) was inserted into the right common carotid artery with caution to minimize unintended surgical damages to the outer wall of the common carotid artery and pushed further into the proximal direction. The balloon then was inflated with water to distend the common carotid artery and pulled back. After this procedure was repeated thrice, the catheter was removed and the insertion port was ligated. The left common carotid artery was kept intact and used as the control. All other procedures including heparin and antibiotic dosing were performed as previously noted.

**Drug Administration**

The beagles were placed randomly into 2 groups. One group (n=5) was orally given tranilast (50 mg/kg BID; Kissei Pharmaceutical) for 2 weeks before balloon injury and for 4 weeks thereafter throughout the experimental period. The other group (n=7) received the vehicle during the same period. Experimental procedures were in accordance with the Guide for the Care and Use of Laboratory Animals (Animal Research Laboratory, Osaka Medical College).

**Histological Analysis**

Four weeks after ballooning, the animals were given an overdose of pentobarbital and exsanguinated by heart excision. The common carotid arteries of both sides were excised and divided into 5 parts, respectively. From each part, a small segment (3 mm long) was obtained and fixed with neutral buffered formalin, paraffin-embedded, and cut into 3-μm-thick sections. The sections were stained with elastica–van Gieson, and cross-sectional areas of intima, media, adventitia, and lumen were quantified with an image analysis system (LUZEX 3; Nikon Optical Co). The neointimal area was traced between the internal elastic lamina (IEL) and the luminal edge of neointima. The medial area was traced between the IEL and the external elastic lamina (EEL). The adventitial area was defined from the EEL outward to the border between the inner dense and the outer loose connective tissues. These measures were the average of 5 different segments for each individual artery. The proliferative activity was assessed by proliferating cell nuclear antigen (PCNA) expression and cell density of each vascular wall compartment. Sections were incubated with primary antibody to PCNA (PC10, 1:150 dilution, DAKO JAPAN) overnight at 4°C and then with a peroxidase-conjugated rabbit anti-mouse immunoglobulin (1:50 dilution, DAKO JAPAN) for 2 hours at 20°C. The number of vasa vasonum in the total adventitial area was counted and expressed as density (vessels/mm²). The number and localization of mast cells were estimated with toluidine blue–stained sections. Because almost all mast cells are located in the adventitia, its density was expressed as total mast cell count in adventitia/total adventitial area. Correlation analyses were performed between (1) intimal and adventitial areas, (2) intimal area and mast cell density, and (3) adventitial area and mast cell density for the injured carotid arteries of vehicle-treated and tranilast-treated dogs.

**Measurement of ACE and Chymase-like Activities**

ACE activity was measured with hippuryl-His-Leu substrate (Peptide Institute). Chymase-like activity was measured with ANG I substrate as described previously.

**Measurement of Plasma Renin Activity and Plasma ANG II Content**

Blood samples for measurement of plasma renin activity (PRA) and plasma ANG II content were taken into chilled tubes containing 5 mmol/L EDTA and 1 mmol/L PMSF (final concentrations) 2 hours after the final dosing. PRA was measured by radioimmunoassay with a commercial kit (SRL). Plasma ANG II content was measured by radioimmunoassay after fractionation by reverse-phase high-performance liquid chromatography, with the use of anti–ANG II antiserum (a gift from Dr S. Kim, Osaka City University).

**Quantitative Reverse-Transcriptase Polymerase Chain Reaction Analysis**

Chymase and ACE mRNA levels of carotid arteries were determined with quantitative reverse-transcriptase polymerase chain reaction (PCR) analysis described previously with modifications. The PCR primers for dog chymase were selected according to the dog chymase cDNA sequence (sense primer: 5'-ATCCTCAGCTCTCCGGAA-TCACCTG-3', position 130 to 153; antisense primer: 5'-CAGAACCCCTTATGTACCAAGGGCCG-3', position 741 to 718). The competitor DNA for dog chymase was obtained by inserting a 360-bp external DNA fragment at the BstEII site. For determination of the dog ACE cDNA sequence, PCR primers were designed at first based on the human ACE cDNA sequence (sense primer: 5'-AACGCCCTGCTAAGCACAATG-3', position 454 to 463; antisense primer: 5'-CATCGAGGGTGGCCGTTCG-3', position 944 to 924). The PCR product was subcloned, and a 504-bp dog ACE cDNA sequence was determined, then the specific PCR primers for quantification of dog ACE transcripts were designed according to the already-determined dog ACE cDNA sequence (sense primer: 5'-AACAGGTACTTCTTCACAGCC-3', position 464 to 484; antisense primer: 5'-AGGCACCACATGTCATAGAG-3', position 919 to 899). The competitor DNA for dog ACE was prepared by inserting a 360-bp external DNA fragment at the BstXI site. The amplification conditions for dog chymase were 94°C for 1 minute, 65°C for 1 minute, and 72°C for 1 minute over 40 cycles. The conditions for dog ACE were 94°C for 1 minute, 60°C for 1 minute, and 72°C for 1 minute over 40 cycles. The integrated density of PCR products was measured as described previously.

**Measurement of Plasma and Vascular Tissue Concentration of Tranilast**

Plasma samples were obtained 2 and 12 hours after drug administration the day before the euthanasia. Plasma aliquots (100 μL) were incubated for 10 minutes at room temperature with 200 μL of internal standard solution (N-cinnamoylalanthranilic acid, 100 μg/mL) and 2 mL of ethanol and centrifuged at 3000 rpm for 10 minutes. Supernatants were evaporated to dryness, and the residues were resuspended with 150 μL of 50% acetonitrile and 10 μL of acetic acid and then fractionated by reverse-phase high-performance liquid chromatography. Common carotid arteries of both sides were dis-
sected 2 hours after the final dosing on the day of euthanasia. The excised arteries (200 mg) were minced and homogenized in 1 mL of 0.02 mol/L NaOH and 200 μL of internal standard solution. The homogenate was incubated for 30 minutes at room temperature with 3 mL of acetonitrile and centrifuged at 3000 rpm for 10 minutes. The supernatant was processed in the same manner as for plasma. The final recovery of tranilast from tissues or plasma after overall procedures was >90%.

Direct Effect of Tranilast on Chymase Activity In Vitro

The direct inhibitory effect of tranilast on the catalytic activity of chymase was analyzed with purified human chymase (from gastro-epiploic arteries24) and dog carotid arterial preparations. One milli-unit of human chymase was preincubated with 10^{-6}, 10^{-5}, 10^{-4}, and 10^{-3} mol/L tranilast in 20 mmol/L Tris-HCl buffer (pH 8.0) containing 0.5 mol/L KCl and 0.1% (vol/vol) Triton X-100 for 1 hour at 37°C and then incubated with 100 μmol/L ANG I for 10 minutes. ANG II was determined as mentioned earlier. After tissue extract from dog carotid artery was preincubated with the same series of concentrations of tranilast for 1 hour at 37°C, its chymase-like activity was measured as described earlier.

Statistical Analyses

All numerical data are expressed as mean±SEM. Significant differences between the means of different groups were evaluated by Student’s t test for unpaired data and by the modified t test for multiple comparison (Tukey’s method) after 2-way ANOVA. Simple linear regression was used to determine correlation between several morphological indexes mentioned in the “Histological Analysis” section.

Results

Effect of Tranilast on Vascular Remodeling

The balloon injury elicited remarkable neointima formation (Figure 1A). Tranilast markedly prevented this neointima formation and decreased the intima/media ratio of injured arteries by 63% (P<0.05, Figures 1B and 2). The lumen area of vehicle-treated injured arteries (1.00±0.09 mm², n=7) was smaller than that of vehicle-treated uninjured arteries (1.56±0.17 mm², n=7, P<0.05), indicating luminal stenosis. After treatment with tranilast, luminal stenosis as well as neointima formation were prevented: The lumen area of injured arteries (1.66±0.21 mm², n=5) was not significantly different from that of uninjured arteries (1.87±0.20 mm², n=5). The cross-sectional area of the media was not altered by the injury, nor differed between the injured arteries of the vehicle-treated and the tranilast-treated dogs. After the injury, fibrosis (Figure 1G), angiogenesis (Figure 1I), and an increase in mast cell count (Figure 1D) were observed in the adventitia. The adventitial area/medial area ratio of injured common carotid arteries from vehicle-treated dog (I) and tranilast-treated dog (J). Scale bar, 100 μm for A through C; 50 μm for D through J.
uninjured arteries (P<0.05, Figure 2). In the tranilast-treated group, adventitia/media ratio did not differ between injured and uninjured arteries (Figure 2). Adventitial fibrosis was analyzed with Azan-stained sections. In vehicle-treated dogs, the blue-stained area in the adventitia of injured arteries (3.82±0.50 mm², n=7) was 2.2 times that of uninjured arteries (1.72±0.20 mm², n=7, P<0.05). In tranilast-treated dogs, the blue-stained area in the adventitia of injured arteries (2.15±0.21 mm², n=5) did not significantly differ from that of uninjured arteries (1.76±0.28 mm², n=5). In vehicle-treated dogs, the vasa vasorum density in injured arteries (0.35%±0.05%, n=5) was significantly lower than that of vehicle-treated injured arteries (0.011%±0.018%, n=5) was significantly lower than that of vehicle-treated injured arteries (0.018%±0.05%, n=7) was significantly lower than that of vehicle-treated injured arteries (0.35%±0.13%, n=7, P<0.05). PCNA indexes in the media, whereas few were found in the media and adventitia. The PCNA index in the neointima of tranilast-treated injured arteries (0.018%±0.011%, n=5) was significantly lower than that of vehicle-treated injured arteries (0.35%±0.13%, n=7, P<0.05). PCNA indexes in the media and adventitia of vehicle-treated injured arteries were <0.05% and did not differ from those of tranilast-treated injured arteries and uninjured arteries. The cell density of each vascular wall compartment was not altered in response to injury, nor differed between the injured arteries of the vehicle-treated and tranilast-treated dogs (Table). There were

few mast cells in uninjured vascular walls, whereas numerous cells were found in the injured vascular wall (Figures 1D and 3A). Mast cells were located primarily in the adventitia and occasionally seen in the outer media (Figure 1D). In the dogs treated with tranilast, mast cell density in the injured arteries was decreased to the level of the uninjured arteries (P<0.05, Figure 3A). Some correlation existed between adventitial and intimal areas (r=0.388, P=0.0026, Figure 3B), between the change in mast cell density and the magnitude of neointima formation (r=0.639, P<0.0001, Figure 3C), and between the change in mast cell density and the magnitude of adventitial thickening (r=0.756, P<0.0001, Figure 3D).

**Chymaselike and ACE Activities of Carotid Arteries and Plasma ACE Activity**

The chymaselike activity of vehicle-treated injured arteries exhibited an increase 10.2-fold that of uninjured arteries (P<0.05). The long-term tranilast treatment completely suppressed the chymaselike activity of injured arteries to the normal level (Figure 4A). The ACE activity of vehicle-treated injured arteries was increased 1.3 times that of uninjured arteries (P<0.05), whereas that of tranilast-treated injured arteries was 1.2 times that of uninjured arteries (P<0.05). Thus tranilast did not affect vascular ACE activity (Figure 4B). The plasma ACE activity of the tranilast-treated dogs (11.2±1.8 mU/mL, n=5) did not differ from that of the vehicle-treated controls (10.5±0.8 mU/mL, n=7).

**PRA and Plasma ANG II Content**

The PRA (0.95±0.20 ng/mL per hour, n=5) and the plasma ANG II content (14.6±6.3 pg/mL, n=5) of tranilast-treated
dogs did not differ from the PRA (0.84±0.38 ng/mL per hour, n=7) and the plasma ANG II content (15.2±3.8 pg/mL, n=7) of vehicle-treated controls.

ACE and Chymase mRNA Levels of Carotid Arteries

The chymase mRNA level of vehicle-treated injured arteries exhibited an increase 4.8-fold that of uninjured arteries (P<0.05). Tranilast treatment blunted the increase of chymase mRNA level in the injured arteries to 57% that for the vehicle-treated group (P<0.05, Figure 5A), although the level still remained significantly higher than that of the uninjured arteries after tranilast treatment (Figure 5A). In contrast, there was no difference in the ACE mRNA levels between the injured and the uninjured arteries in vehicle-treated dogs (Figure 5B). Tranilast did not affect the ACE mRNA levels of injured or uninjured arteries (Figure 5B).

Plasma and Tissue Tranilast Concentrations

Plasma tranilast concentrations 2 and 12 hours after its dosing on the penultimate day of the experiment were 297±33 μmol/L and 55.0±9.2 μmol/L, respectively. Tissue tranilast concentration 2 hours after the final dosing was 32±7 nmol/g tissue in injured carotid arteries and 29±3 nmol/g tissue in uninjured arteries.

Direct Effect of Tranilast on Chymase Activity In Vitro

Tranilast at concentrations of 10⁻⁶, 10⁻⁵, 10⁻⁴, and 10⁻³ mol/L inhibited the ANG II-forming activity of purified human chymase by 2%, 4%, 7%, and 25%, respectively. Tranilast in concentrations up to 10⁻³ mol/L did not inhibit the chymaselike activity in the tissue extract of dog carotid artery.

Discussion

In the injured arteries, chymaselike activity and chymase mRNA level were remarkably increased, whereas a slight increase in ACE activity and no increase in ACE mRNA expression were detected. The current study demonstrated for the first time that tranilast prevented vascular chymase expression and effectively inhibited neointima formation and luminal stenosis. Our previous study showed that the vascular ANG II content doubled in the injured arteries compared with that in the uninjured arteries and that an ANG II receptor antagonist but not an ACE inhibitor prevented the neointima formation.¹⁵ In the current study, tranilast treatment did not affect plasma ANG II concentration, plasma ACE activity, or PRA. Vascular ANG II–forming activity of chymase increased 4.8-fold in vehicle-treated dogs, whereas tranilast treatment completely prevented the increase in chymase activity. Therefore, the ANG II–forming rate in local vascular tissues supposedly increased after vascular injury but returned to the normal level by tranilast treatment. Tranilast decreased significantly the intima/media ratio and the PCNA index of the neointima. Tranilast may exhibit an antiproliferative effect on neointimal cells. These results suggest that the activated vascular chymase promotes tissue remodeling by augmenting the local ANG II production in injured vascular tissues.

Long-term treatment with tranilast completely suppressed the increase of vascular chymaselike activity in vivo. However, tranilast up to a concentration of 10⁻³ mol/L did not directly affect the chymaselike activity in vitro. Chymase derives mainly from connective tissue–type mast cells. In the current study, mast cell density was markedly increased in the balloon-injured arteries, but such an increase was almost completely prevented by the long-term tranilast treatment. A clinical finding also showed that the mast cell counts were reduced in lesions of urticaria pigmentosa after long-term tranilast treatment.²⁵ Mast cell count was greatly increased in the adventitia after balloon injury, but few PCNA-positive cells were there, indicating that the mast cell number might be increased primarily because of migration into the adventitia. Therefore tranilast might inhibit the mast cell migration, thereby indirectly suppressing vascular chymaselike activity. Cell-to-cell interaction between fibroblasts and mast cells through regulation of stem cell factor (SCF)/c-kit expression is essential for the development of mast cells. Tranilast is also known to downregulate the activity of fibroblasts.¹⁶,¹⁹ Although the precise mechanism is yet unknown, tranilast may inhibit the development as well as degranulation of mast cells.

In addition to neointima formation, adventitial thickening occurred in the injured arteries. PCNA-positive cells were
found mainly in the neointima, whereas few were found in the adventitia. A recent study with pig balloon-injured models indicated that the PCNA index of each vascular wall compartment increased maximally 3 to 7 days after injury and returned to baseline after 28 days. Our study analyzed the proliferative activity of arteries 4 weeks after balloon injury, when the adventitia might become largely quiescent. Furthermore, adventitial cell density of injured arteries did not differ from that of uninjured arteries, whereas Azan-stained areas of collagen fibers increased in the adventitia of injured arteries. These results suggest that adventitial thickening can be ascribed mainly but not simply to collagen accumulation. Tranilast prevented adventitial fibrosis as well as neointimal hyperplasia. Recent observations indicated the close interaction between adventitial remodeling and neointima formation. Neointima developed also after direct adventitial manipulation alone without any endoluminal vascular injury. Indeed, the current study showed the correlation between intimal and adventitial thickening. Vascular adventitia consists of a variety of cells and structures, of which we focused on the development of vascular mast cells as the main source of chymase. In the adventitia, mast cell density was increased remarkably, with which the degree of neointima formation was closely correlated. Our results are supported by the others showing that mast cell counts were increased in the adventitia of the aorta and coronary artery with atherosclerotic lesions. The current study also showed the proliferation of vasa vasorum in the adventitia. Another report has demonstrated that perivascular angiogenesis is correlated with the degree of intimal hyperplasia. These findings suggest that various growth factors including ANG II which are formed in the adventitia may be delivered to the media and intima through vasa vasorum.

Tranilast has been shown to display diverse biological actions in vitro. It inhibits (1) migration and proliferation of vascular SMCs induced by platelet-derived growth factor and transforming growth factor-β (TGF-β), (2) collagen synthesis by vascular SMCs and fibroblasts, (3) release of TGF-β from fibroblasts, and (4) fibroblast proliferation. These cellular effects of tranilast occurred with concentrations from $3 \times 10^{-5}$ to $3 \times 10^{-4}$ mol/L. Furthermore, a recent study indicated that tranilast inhibited the ANG II binding to its type 1 receptors, with an IC$_{50}$ of $2.9 \times 10^{-5}$ mol/L. In the current study, plasma tranilast concentrations 2 and 12 hours after the final dosing were 2.97$ \times 10^{-4}$ mol/L and 5.5$ \times 10^{-5}$ mol/L, respectively, whereas tissue tranilast contents of injured and uninjured carotid arteries at 2 hours were $\approx 3 \times 10^{-8}$ mol/g tissue. Thus although plasma tranilast concentrations reached the levels to exhibit many biological actions up to 12 hours, its concentrations in carotid arterial tissues scarcely reached the minimum effective level ($3 \times 10^{-5}$ mol/L) at 2 hours when the plasma concentration reached the peak. Although we could not exclude the possibility that tranilast might exert its beneficial effects for preventing intimal thickening by diverse actions described above, a dose of $> 50$ mg/kg (BID) is required to exhibit those actions sufficiently in vivo. Instead, tranilast at the dose of 50 mg/kg completely inhibited the increases in mast cell numbers and ANG II–forming chymase activity in the injured vascular walls. Accordingly, inhibition of chymase-dependent ANG II–forming pathway by suppressing both activation and development of vascular mast cells may be the most likely mechanism for exhibiting the beneficial effects of tranilast. A recent preliminary clinical trial indicated that tranilast (600 mg/d) treatment for 3 months effectively prevented restenosis after PTCA. After an oral dose of 600 mg/d, the maximum plasma concentration of tranilast was $10^{-4}$ mol/L, which was near the levels found in the current dog study. Further clinical trials of tranilast are awaited to clarify the pathogenesis of restenosis and to introduce new strategies for prevention of restenosis by antiallergic drugs.

In the current study, treatment with tranilast (50 mg/kg BID) reduced the intima/media ratio of carotid arteries by 63%, which was comparable to 67% reduction by the maximum effective dose (10 mg/kg BID) of an AT$_1$ receptor antagonist, TCV-116 (our unpublished data). Thus tranilast exhibited a strong beneficial effect comparable to the maximum dose of ANG II receptor antagonist. Although both drugs are involved in the inhibition of ANG II actions, their fundamental mechanisms of the beneficial effect may differ from each other. To clarify the true mechanism of tranilast, further studies are needed for the combined effects of these 2 agents.

In addition to chymase, mast cells express growth factors (fibroblast growth factor [FGF-2], TGF-β, and so on) and proteases (tryptase and so on). FGF-2 is well known to be synthesized in fibroblasts, macrophages, and endothelial cells, whereas recent observations indicate that mast cells are a major source of FGF-2 in skin disorders characterized by fibrosis and angiogenesis. Furthermore, dog mastocytoma cells produce TGF-β, which promotes deposition of extracellular matrix and is associated with fibrotic conditions. The pathophysiological role of tryptase, another major secretory product of mast cells, remains unclear, but recent studies proved tryptase as a potent mitogen for fibroblasts. Tranilast completely inhibited the injury-induced increase of mast cells. Therefore it is possible that tranilast treatment may suppress these potent fibroproliferative factors together with chymase. The current study explored the beneficial effect of tranilast from the aspect of inhibition of chymase-dependent ANG II–forming pathway. However, in view of the overall functions of vascular mast cells, tranilast is potentially more beneficial than a simple ANG II receptor antagonist.

Acknowledgments

This work was supported in part by grants-in-aid for encouragement of young scientists No. 07770073 and No. 08770076 (to Dr. Shiota in 1995 and 1996) and a grant-in-aid for scientific research (No. 07670107 to Dr. Okunishi in 1995) from the Ministry of Education, Science, Sports, and Culture, Japan.

References

Vascular Chymase and Neointima Formation


Tranilast Suppresses Vascular Chymase Expression and Neointima Formation in Balloon-Injured Dog Carotid Artery
Naotaka Shiota, Hideki Okunishi, Shinji Takai, Imao Mikoshiba, Hiroshi Sakonjo, Nobuo Shibata and Mizuo Miyazaki

_Circulation_. 1999;99:1084-1090
doi: 10.1161/01.CIR.99.8.1084

_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1999 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/99/8/1084

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Circulation_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Circulation_ is online at:
http://circ.ahajournals.org//subscriptions/