

# Curcumin Inhibits Platelet-Derived Growth Factor–Stimulated Vascular Smooth Muscle Cell Function and Injury-Induced Neointima Formation

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**Objective**—Vascular smooth muscle cell (VSMC) migration, proliferation, and collagen synthesis are key events involved in the pathogenesis of cardiovascular disease. Growth factors, such as platelet-derived growth factor (PDGF) and fibroblast growth factor, released during vascular injury plays a pivotal role in regulating these events. Curcumin (diferuloyl methane), a major component of the spice turmeric (*Curcuma longa*), has been shown recently to have beneficial effects in chronic conditions, such as inflammation, cancer, cystic fibrosis, and Alzheimer's disease. The objective of this study was to investigate the ability of curcumin to inhibit PDGF-stimulated migration, proliferation, and collagen synthesis in cultured VSMCs and neointima formation after carotid artery injury in rats.

**Methods and Results**—Curcumin (1 to 25  $\mu\text{M}$ ) produced a concentration-dependent inhibition of PDGF-elicited VSMC migration, proliferation, and collagen synthesis assessed by chemotaxis, [ $^3\text{H}$ ]thymidine incorporation, and [ $^3\text{H}$ ]-L-proline incorporation, respectively. Curcumin blocked PDGF-induced VSMC actin-cytoskeleton reorganization, attenuated PDGF signal transduction, and inhibited the binding of PDGF to its receptors. Carotid artery neointima formation was significantly attenuated by perivascular curcumin compared with vehicle controls 14 days after injury, characterized by reduced DNA synthesis, collagen synthesis, and PDGF receptor phosphorylation.

**Conclusions**—These data suggest that curcumin is a potent inhibitor of key PDGF-stimulated VSMC functions and may play a critical role in regulating these events after vascular injury. (*Arterioscler Thromb Vasc Biol.* 2006;26:85-90.)

**Key Words:** curcumin ■ neointima ■ platelet-derived growth factor

The primary event in the development of atherosclerosis and restenosis after percutaneous transluminal coronary angioplasty is thought to involve injury to the endothelium, leading to a response that may be similar to wound healing requiring migration of vascular smooth muscle cells (VSMCs) from the media to the intima and subsequent proliferation.<sup>1-3</sup> Intimal smooth muscle cells (SMCs) in the intima assume a synthetic phenotype vis-à-vis the normal contractile phenotype, resulting in the deposition of extracellular matrix within the neointimal tissue.<sup>1,2</sup> Although the mechanisms responsible for migration and proliferation of VSMCs are not fully understood, several factors produced in response to vascular injury have been implicated in this process.<sup>3</sup>

Platelet-derived growth factor (PDGF) is a potent growth factor produced by platelets, VSMCs, and endothelial cells in the injured vascular wall.<sup>4,5</sup> PDGF initiates a multitude of biological effects through the activation of intracellular signal transduction pathways that contribute to VSMC proliferation, migration, and collagen synthesis.<sup>6</sup> The importance of PDGF

in the development of neointima has been established in arterial injury models.<sup>6</sup> PDGF is also a potent stimulant of extracellular matrix synthesis by VSMCs. Accordingly, inhibition of PDGF-stimulated VSMC migration, proliferation, and extracellular matrix synthesis represents an important point of therapeutic intervention to attenuate cellular manifestations of many vascular diseases.

Curcumin [Figure 1; diferuloyl methane; 1,7-bis(4-hydroxy-3-methoxy-phenyl)-1,6-heptadiene-3 to 5-dione], the major yellow pigment extracted from turmeric (the powdered rhizome of the herb *Curcuma longa*), has been used in indigenous medicine to treat a variety of inflammatory conditions and chronic diseases<sup>7</sup> and is commonly used as a coloring and flavoring additive in foods. Recent studies indicate that dietary administration of curcumin may have beneficial effects in conditions such as cancer,<sup>8</sup> Alzheimer's disease,<sup>9</sup> and cystic fibrosis.<sup>10</sup> With regard to mode of action, curcumin exhibits a diverse array of metabolic, cellular, and molecular activities. Our earlier studies have demonstrated

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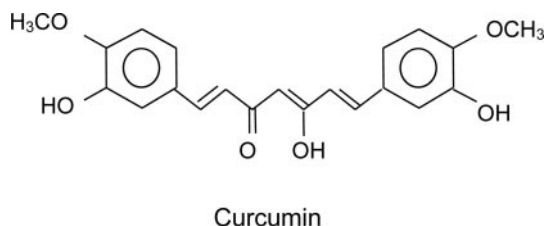
From the Division of Pharmaceutical Sciences and Center for Cardiovascular Research and Alternative Medicine (X.Y., X.Z., B.W.C., J.R., N.S.), the Division of Kinesiology and Health (D.P.T.), and the Department of Animal Sciences (B.M.A., W.J.M.), University of Wyoming, Laramie; Divis Laboratories Limited (M.N.A.R.), Hyderabad, India; and J.L. Chambers Biomedical/Biotechnology Research Institute (D.A.T.), North Carolina Central University, Durham.

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**Figure 1.** Chemical structure of curcumin [1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3 to 5-dione].

that curcumin has potent antioxidant properties.<sup>11</sup> Curcumin has been demonstrated to be a potent inhibitor of the oxidant stress-induced transcription factor nuclear factor- $\kappa$ B,<sup>12</sup> activator protein-1,<sup>13</sup> and the Janus kinase–signal transduction activating transcription pathway (JAK-STAT) signaling pathway.<sup>14</sup> These signaling molecules are known regulators of inflammation and cell proliferation, which represent key features of the response to vascular complications.

Based on the above considerations, the purpose of the present investigation was to determine the effects of curcumin on PDGF-stimulated VSMC migration, proliferation, and collagen synthesis, as well as the intracellular mechanism(s) of these actions. Furthermore, we also assessed the effect of curcumin on neointima formation after balloon injury in rats.

## Methods

### Curcumin

Commercial curcumin contains amounts of the isomers desmethoxycurcumin and bisdemethoxycurcumin, thus rendering it impure; hence, pure curcumin (Figure 1) was synthesized by condensing vanillin with acetyl acetone as a boron complex.<sup>15</sup> The purity and the chemical structure were confirmed by melting point, elemental analysis, and spectral studies.

### SMC Isolation and Culture

Thoracic aorta SMCs were obtained from male Sprague-Dawley rats weighing between 100 and 200 g, as described previously.<sup>16</sup> All of the experiments were performed using early cell passages<sup>2–4</sup> of VSMCs. This study was performed via a protocol approved by the Institutional Animal Care and Use Committee of the University of Wyoming in accordance with the Guide for the Care and Use of Laboratory Animals.

### Cell Migration Assay

Two cell migration assays were used. In the first, modified Boyden transwell chambers were used for monitoring cell migration as described by us previously.<sup>17</sup> Cells (35 000 cells/well) were seeded onto the apical (upper) chamber of the transwell, and the lower chamber contained the experimental reagents. Cells were allowed to migrate for 6 hours, after which the inserts were removed. Nonmigrating cells in the upper chamber were removed, and cells in the bottom membrane were fixed with 3.7% formaldehyde for 10 minutes and stained with 0.4% hematoxylin for 5 minutes. The number of migrated cells was measured by counting the number of stained nuclei from 4 randomly chosen high-power ( $\times 400$ ) fields.

In the second assay, migration was measured using a monolayer-wounding protocol in which cells migrated from a confluent area into an area that was mechanically denuded of cells.<sup>16</sup> The area of migration was photographed with a video camera system using Scion Image software at the intersection of the previously marked line and wound edge at 0 hours ( $WW_0$ ) and at 24 hours ( $WW_{24}$ ) after treatment with PDGF (10 ng/mL) in the presence or absence of curcumin (1 to 25  $\mu$ M). Cell migration was calculated as wound width covered at time  $t$  ( $WW_t - WW_{24}$ ) and expressed as the percentage of control.

### Staining of F-Actin With Rhodamine Phalloidin

VSMCs were grown in Nunc chamber slides until  $\approx 60\%$  confluence. After serum deprivation for 48 hours, cells were incubated at 37°C for 1 hour with the experimental medium containing or lacking curcumin (10  $\mu$ M) in the presence or absence of PDGF (10 ng/mL). Cells were fixed and rendered permeable with 3.7% formaldehyde:2% Triton X-100, washed with phosphate-buffered saline (containing 1% BSA), incubated with rhodamine phalloidin,<sup>18</sup> and examined by fluorescence microscopy (Nikon TE300 inverted microscope equipped with a Cascade 650 cooled CCD digital camera,  $\times 100$  magnification).

### [<sup>3</sup>H]Thymidine Incorporation Assay

Cell proliferation was assessed by [<sup>3</sup>H]thymidine incorporation in mitogenically quiescent VSMCs.<sup>17</sup> Cells were incubated for 18 hours with or without of PDGF (10 ng/mL) and various concentrations of curcumin and then pulse-labeled with 1  $\mu$ Ci/mL of [<sup>3</sup>H]thymidine for 6 hours. Cells were washed 3 times with PBS, precipitated with 10% (wt/vol) ice-cold trichloroacetic acid, and rinsed with absolute ethanol and air dried. For analysis, the monolayer was dissolved in 250  $\mu$ L of 0.5 mol/L NaOH per well at room temperature overnight. Duplicate samples of 100  $\mu$ L were counted in scintillation fluid in a liquid scintillation counter (Beckmann LC 6000IC). A second aliquot was used for the determination of protein content via the Bradford assay (BioRad Laboratories Inc) per the manufacturer's specifications.

### Collagen Synthesis

Collagen synthesis was determined by measuring [<sup>3</sup>H]-L-proline incorporation.<sup>19</sup> Collagen synthesis was initiated by treating quiescent VSMCs with [<sup>3</sup>H]-L-proline (5  $\mu$ Ci/mL) in the presence or absence of PDGF and curcumin. Cells were processed in a similar manner as that described for the proliferation assay for determining the radioactivity.

### SDS-PAGE and Immunoblotting

Western blotting for protein analysis was performed as described previously.<sup>16</sup> Cells were lysed in radioimmunoprecipitation assay buffer containing 1  $\mu$ M sodium vanadate, 1  $\mu$ M phenylmethylsulfonyl fluoride, 5  $\mu$ g/mL aprotinin, and 5  $\mu$ g/mL leupeptin. Protein concentration was determined by the bicinchoninic acid method (Pierce Biotechnology Inc). Lysates corresponding to equal amounts of proteins were boiled in Laemmli sample buffer, and the supernatants were loaded onto gels for SDS-PAGE. Proteins were transferred onto nitrocellulose membranes and probed with the following primary antibodies: anti-phospho-PDGF receptor (1:1000), anti-PDGF receptor  $\beta$  (1:1000), antiphospho-extracellular signal regulated kinase (Erk)1/2 (1:3000), anti-Erk (1:5000), antiphospho insulin receptor  $\beta$ /insulin-like growth factor receptor (1:1000), and anti-phospho Akt (Thr308). Appropriate horseradish peroxidase-coupled secondary antibodies were used at 1:10 000. Immunoreactive bands were visualized using Renaissance chemiluminescence reagents. Films were scanned, and the intensity of immunoblot bands was detected with a BioRad Calibrated Densitometer (Model: GS-800). For Western blotting of carotid arteries, whole tissues were harvested and stored at  $-80^\circ\text{C}$ . Tissue lysates were made by homogenizing and sonicating the samples in radioimmunoprecipitation assay buffer.

### PDGF Receptor Binding Assay

The receptor binding assay was performed as described previously.<sup>20</sup> Confluent monolayer of cells cultured in 24-well culture plates at a density of  $5 \times 10^4$  cells per well were rendered quiescent and incubated with 0.05 nM [<sup>125</sup>I]-PDGF-BB (Amersham Pharmacia) in the presence or absence of curcumin at 4°C for 2 hours. After washing, the cells were lysed in 25  $\mu$ M HEPES-NaOH buffer (pH 7.4) containing 1% Triton X-100 and 10% glycerol, and cell-associated radioactivity was measured in a gamma counter (Packard, Model 5005). Nonspecific binding was determined in the presence of 1000 ng/mL of unlabeled PDGF-BB. Specific binding was determined by subtracting the nonspecific binding from the total binding.

### Rat Carotid Artery Balloon Injury

The procedure for balloon injury in rat carotid arteries has been described previously.<sup>21</sup> Briefly, male Sprague-Dawley rats (450 to 500 g) were anesthetized with an IP injection of 100 mg/kg ketamine and 5 mg/kg of xylazine. The left carotid artery was isolated, and a Fogarty 2F embolectomy catheter (Edward life Sciences) was introduced through the external carotid arteriotomy incision, advanced to the aortic arch, inflated to produce moderate resistance, and gradually withdrawn 3 times. The catheter was removed, the external carotid branch ligated, blood flow through the common carotid verified, and 200  $\mu$ L of Pluronic gel containing curcumin 72  $\mu$ g (obtained by dissolving 100- $\mu$ L stock solution of 10  $\mu$ M curcumin in DMSO with 900  $\mu$ L of the gel) or 25% Pluronic gel containing vehicle (DMSO) was applied to the exposed adventitial surface of the injured artery in a random manner. The incision was closed, and buprenorphine (0.1 mg/kg) was administered intraperitoneally. On full recovery from anesthesia, rats were returned to the animal care facility and provided standard rat chow and water ad libitum. Sham-operated control experiments with/without curcumin were performed in a similar fashion to analyze surgery-associated effects on arterial remodeling without the influence of balloon injury.<sup>22</sup> At specific times, rats were overdosed and euthanized via pneumothorax and exsanguination. For histology, tissues were perfusion-fixed with PBS and buffered formalin phosphate, and the carotids were harvested and processed. For Western blots, tissues were freshly removed and snap-frozen. All of the animal protocols complied strictly with the Institutional Animal Care and Use Committee guidelines.

### Tissue Processing, Staining, and Morphometric Analyses

Tissues were processed in graded alcohols and xylenes and paraffin-embedded using standard staining procedures.<sup>21</sup> Microscopic analyses and quantification of morphological parameters were performed using Zeiss AxioVision 4.3 (Carl Zeiss) and Adobe Photoshop 5.5 (Adobe Systems) software systems linked through a Zeiss Axiocam MR color digital camera to a Zeiss Axioskop 2 Plus light microscope. Images were captured and quantitative measurements made in a blinded manner of perimeters and areas corresponding to internal and external elastic laminae and the lumen. Data transformations provided data for neointimal and medial wall areas and vessel diameters.

### Proliferating Cell Nuclear Antigen Immunohistochemistry

Standard immunohistochemical techniques were used with an anti-proliferating cell nuclear antigen (PCNA) monoclonal antibody (1:25) and a biotinylated antimouse secondary antibody (1:100) on perfusion-fixed, paraffin-embedded tissues.<sup>22</sup> Slides were treated with avidin-biotin block and exposed to diaminobenzidine black chromogen with nuclear fast red counterstain. Slides were cover-slipped for analysis under light microscopy. Data are represented as PCNA labeling index defined as the percentage of total medial wall cells positive for PCNA.

### Release of Curcumin From Pluronic Gel

Curcumin was dissolved in Pluronic gel and placed in *n*-octanol and incubated at 37°C. The aliquot of octanol was removed at different times, and the concentration of curcumin was determined spectrophotometrically at 434 nm as reported previously.<sup>15</sup>

### Data Analysis

Statistical significance was determined by using the unpaired Student *t* test or 1-way ANOVA with the Fisher multiple comparison test. All of the data are expressed as mean  $\pm$  SEM. A *P* value of <0.05 is considered statistically significant for all of the comparisons.

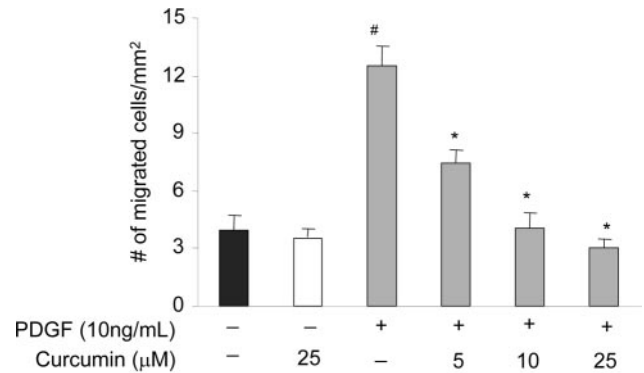


Figure 2

**Figure 2.** Curcumin attenuates PDGF-stimulated smooth muscle cell motility in a transwell migration assay. Treatment with curcumin significantly reduced the number of cell migration in response to PDGF. Results are the mean  $\pm$  SE from 5 independent experiments. #*P* < 0.005 compared with control; \**P* < 0.01 compared with PDGF-stimulation.

## Results

### Curcumin Inhibits PDGF-Stimulated VSMC Migration

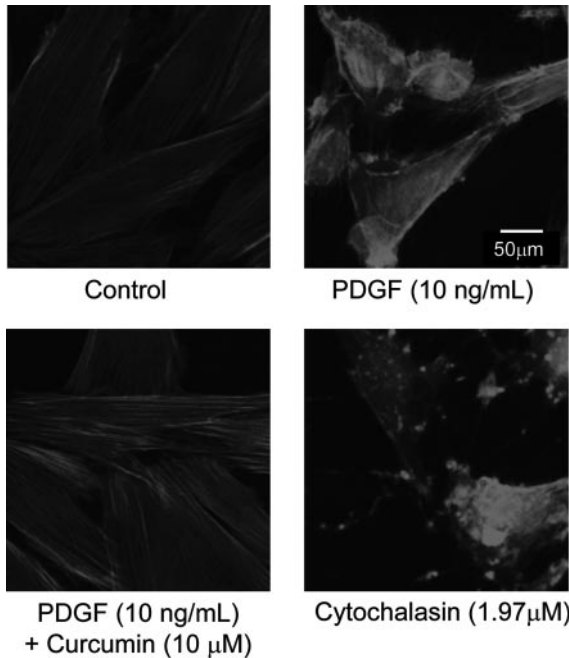
In the transwell migration assay (Figure 2) that represents chemotaxis, PDGF (10 ng/mL) enhanced the basal migration of VSMCs by  $\approx$ 4-fold (basal migration 3.2  $\pm$  0.31 cells/mm<sup>2</sup> versus 12.4  $\pm$  1.5 cells/mm<sup>2</sup> after PDGF stimulation). Treatment with curcumin (1 to 25  $\mu$ M) resulted in concentration-dependent inhibition of cell migration with statistical significance achieved at 5  $\mu$ M (7.4  $\pm$  0.53 cells per mm<sup>2</sup>; *P* < 0.005) with virtually complete inhibition achieved at 25  $\mu$ M. Similar results were obtained in the cell monolayer wounding assay that represents chemokinesis (please see Figure I, available online at <http://atvb.ahajournals.org>). Treatment with PDGF for 24 hours caused cells to migrate to 64.3  $\pm$  9.4% of the wound width. In the presence of 25  $\mu$ M of curcumin, the cells migrated to cover only 19.4  $\pm$  6.8% of the wound width, which was similar to the migration observed in untreated cells (19.1  $\pm$  5.4%).

### Curcumin Inhibits PDGF-Stimulated Cytoskeletal Reorganization

As shown in Figure 3, PDGF (10 ng/mL) caused significant actin filament disassembly, consistent with previous observation.<sup>23</sup> PDGF-stimulated dissociation of actin filament was blocked by curcumin (10  $\mu$ M) and mimicked by the positive control cytochalasin.

### Curcumin Inhibits PDGF-Stimulated VSMC Proliferation

In the [<sup>3</sup>H]thymidine incorporation assay (Figure 4), stimulation with PDGF (10 ng/mL) increased cell proliferation by  $\approx$ 4-fold. Curcumin (1 to 25  $\mu$ M) inhibited PDGF-stimulated cell proliferation in a concentration-dependent manner with 76% inhibition observed at 25  $\mu$ M. When quiescent cells were treated with curcumin (1 to 25  $\mu$ M) for 24 hours in the absence of PDGF, no significant difference was observed in the extent of [<sup>3</sup>H]thymidine incorporation, suggesting that curcumin is not cytotoxic at

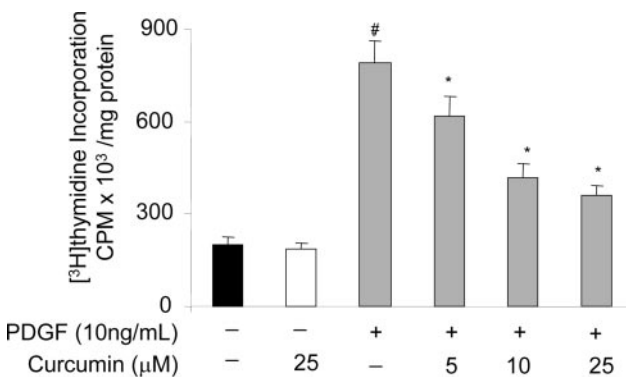


**Figure 3.** Curcumin inhibits PDGF-induced dissociation of actin microfilaments. Cells grown on slides were incubated with medium containing or lacking curcumin (10  $\mu$ M) in the presence or absence of PDGF (10 ng/mL) for 1 hour. After fixing and permeabilization, cells were stained with rhodamine phalloidin and examined by fluorescence microscopy. Cytochalasin was used as a positive control.

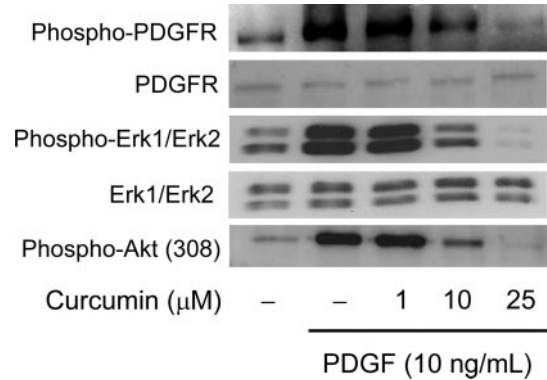
the concentrations tested. The lack of cytotoxicity of curcumin at the concentrations used in these experiments was also ascertained by the trypan blue exclusion assay (data not shown). Furthermore, at equimolar concentrations, curcumin exhibited poor inhibition of VSMC proliferation induced by basal fibroblast growth factor (bFGF) and insulin, suggesting that the effects of curcumin on PDGF-stimulated proliferation may be fairly specific (please see Table I, available online at <http://atvb.ahajournals.org>).

### Curcumin Inhibits PDGF-Stimulated Collagen Synthesis

Curcumin (1 to 25  $\mu$ M) inhibited PDGF-stimulated [ $^3$ H]-L-proline incorporation in a concentration-dependent manner



**Figure 4.** Curcumin inhibits PDGF-stimulated smooth muscle cell proliferation. Cells were treated with PDGF (10 ng/mL) for 24 hours in the presence or absence of curcumin, and incorporation of [ $^3$ H]thymidine was monitored and normalized to protein. Results are the mean  $\pm$  SE from 5 independent experiments. <sup>#</sup> $P < 0.005$  compared with control; <sup>\*</sup> $P < 0.01$  compared with PDGF-stimulation.



**Figure 5.** Treatment with curcumin inhibits PDGF-stimulated phosphorylation of PDGF receptor  $\beta$ , Erk1/2, and Akt. Cells were treated with or without (control) various concentrations of curcumin (0.1 to 25  $\mu$ M) for 30 minutes in the presence or absence of PDGF (10 ng/mL) for 10 minutes. Cells were lysed and lysates immunoblotted with antibodies directed against phospho-PDGFR receptor, phospho-Erk1/2, and phospho-Akt. Loading of equivalent amounts of protein was confirmed by stripping the membranes and reprobing with antibodies directed against PDGF-receptor  $\beta$  and Erk1/2.

with complete inhibition observed at 25  $\mu$ M (please see Figure II, available online at <http://atvb.ahajournals.org>).

### Curcumin Attenuates PDGF-Stimulated Tyrosine Phosphorylation of PDGF-Receptor $\beta$ , Erk1/2, and Akt

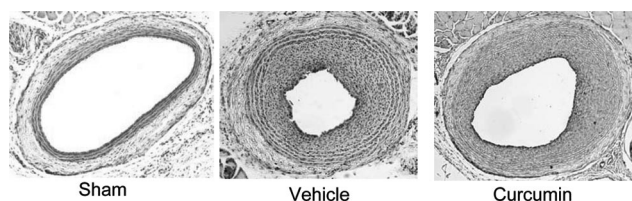
As shown in Figure 5, PDGF increased phosphotyrosine levels on PDGF-receptor  $\beta$  by  $\approx$ 4-fold, whereas curcumin blocked this effect in a concentration-dependent manner. Neither treatment with PDGF nor curcumin changed the protein levels of the PDGF receptor. In a similar manner, curcumin blocked the capacity of PDGF to stimulate the phosphorylation of downstream effectors Erk1/Erk2 and Akt (Figure 5). Figure III (available online at <http://atvb.ahajournals.org>) gives the ratio of the densities of phosphoproteins to total proteins for the blots discussed here. In contrast to its effects on PDGF signal transduction, curcumin failed to attenuate insulin-stimulated phosphorylation of insulin receptor  $\beta$ /insulin-like growth factor receptor and bFGF-stimulated phosphorylation of phosphofibroblast growth factor receptor substrate 2a (Figure IV, available online at <http://atvb.ahajournals.org>).

### Curcumin Inhibits PDGF Receptor Binding

As shown in Figure V (available online at <http://atvb.ahajournals.org>), curcumin inhibited the binding of [ $^{125}$ I]-PDGF-BB to VSMC surface receptors in a concentration-dependent manner. The  $IC_{50}$  of the binding was 23.2  $\mu$ M.

### Curcumin Inhibits Neointima Formation

Representative photomicrographs of balloon-injured, perfusion-fixed left carotid artery cross-sections 14 days after injury are shown in Figure 6, including an untreated section from a sham surgery, a section of injured artery exposed to Pluronic gel containing the vehicle, and a section of the injured artery exposed to curcumin. Figure 6 and Figure VI (available online at <http://atvb.ahajournals.org>) illustrates that the neointima:media ratio is significantly reduced ( $\approx$ 40%) by



**Figure 6.** Representative photomicrographs (magnification  $\times 100$ ) of balloon-injured, perfusion-fixed, and crystal violet-stained left carotid arteries 14 days after injury. Curcumin-treated arteries exhibit significant reduction of neointima formation compared with vehicle-treated arteries.

curcumin ( $72 \mu\text{g}$ ) compared with gel controls. The lower concentration of curcumin ( $7.2 \mu\text{g}$ ), however, did not significantly inhibit the injury-induced neointima formation (results not shown). In vitro release studies showed that Pluronic gel imparts a sustained release property to curcumin ( $\approx 60\%$  released in 72 hours) without deleteriously affecting its chemical nature (data not shown).

Online Figure VII (available online at <http://atvb.ahajournals.org>) shows that curcumin inhibits DNA synthesis (PCNA immunostaining) and attenuates protein levels of the phospho-PDGF receptor and collagen in arteries 72 hours after injury, complimenting the attenuation of neointima observed in the curcumin-treated vessels.

## Discussion

PDGF plays an important role in vascular remodeling during cellular and extracellular response to injury.<sup>4,6,24</sup> Whereas the PDGF receptor is expressed at low levels in arteries from healthy adults, its expression is upregulated during endothelial injury after angioplasty or in the early stage of atherosclerosis.<sup>4</sup> PDGF has also been implicated in neointima formation after angioplasty and in posttransplant arteriopathy.<sup>6,24</sup> Targeting PDGF with an anti-PDGF antibody,<sup>25</sup> receptor expression with antisense oligonucleotides,<sup>26</sup> or using inhibitors of PDGF signal transduction<sup>27</sup> have all been shown to attenuate neointima development. We report here that curcumin, the major component of the food-additive turmeric, has potent and dose-dependent inhibitory effects on PDGF-stimulated VSMC proliferation, migration, and collagen synthesis. We have also shown using an animal arterial balloon-injury model characterized by PDGF-receptor upregulation<sup>4</sup> that curcumin significantly inhibits neointima development, collagen accumulation, and PDGF-receptor phosphorylation. Based on the inhibition by curcumin of both the PDGF-stimulated Erk and Akt signaling pathways, it seems reasonable to conclude that these inhibitory effects are at least in part attributable to inhibition of PDGF signal transduction.

PDGF binds to its cognate receptor tyrosine kinase inducing the autophosphorylation of tyrosine residues within the cytoplasmic domain of the receptors and resulting in the recruitment and activation of specific signaling molecules that may mediate the migration and proliferation of VSMCs in response to injury.<sup>6</sup> It has been suggested that different signaling pathways regulate the proliferative compared with the migratory responses to PDGF, with mitogen-activated protein kinase regulating proliferation and phosphatidylinositol 3-kinase regulating migration.<sup>28</sup> Recent

studies have indicated that the inhibition of monocyte chemotaxis by curcumin may be mediated via modulation of growth response-1 transcription factor.<sup>29</sup> The ability of curcumin to inhibit PDGF-stimulated Erk and Akt phosphorylation suggests that curcumin may be mediating its effects on PDGF signaling by acting upstream of the nuclear events. Nonetheless, curcumin also inhibited the basal phosphorylation of Erk1/2 (Figure VIII, available online at <http://atvb.ahajournals.org>). However, the lack of inhibition by curcumin of the insulin and bFGF-stimulated proliferation (which also work through the Erk pathway) suggests that dephosphorylation of Erk under basal conditions may not be playing a major role in this process.

Phosphorylation in cells is dynamically regulated by kinases and phosphatases. Previous studies have shown that an aqueous extract of *Zedoarie rhizoma* (which has curcumin as one of its components) inhibits the binding of PDGF to its receptors.<sup>20</sup> Our results support this finding: curcumin inhibited the binding of radiolabeled PDGF to its receptor on the cell surface (Figure V) in a concentration-dependent manner. However, the  $\text{IC}_{50}$  value for this inhibition was higher than that required for the dephosphorylation of the PDGF receptor ( $23.8 \mu\text{M}$  versus  $9.4 \mu\text{M}$ , respectively) indicating the involvement of other mechanisms as well.

Neointima-induced vessel stenosis after percutaneous angioplasty is a major clinical complication, occurring in 30% to 50% of patients within 3 to 6 months of the procedure.<sup>30</sup> In this study, a well-established carotid injury model was used to investigate the ability of curcumin to protect against the neointima response to vascular injury. Curcumin significantly attenuated neointima formation, cell proliferation, collagen synthesis, and the overexpression of PDGF receptors after vascular injury in the rat carotid vasculature. These results substantiate our in vitro findings and strongly support the notion that curcumin confers protection against injury-induced pathological remodeling of blood vessels.

Various attempts have been made to regulate the migratory, proliferative, and synthetic phenotype of VSMCs in a cumulative effort to minimize vascular injury and neointima development.<sup>25,26</sup> Inhibitors of PDGF signaling via varied mechanisms have been shown to successfully combat the pathological processes associated with restenosis. Clinical trials using the rather nonspecific PDGF-inhibitor trapidil demonstrated the utility of this approach in diminishing the response to vascular injury.<sup>31</sup> Drugs such as imatinib mesylate, now used clinically as an antitumor agent, were developed with the primary goal of preventing restenosis after coronary angioplasty through the inhibition of abnormal PDGF receptor activation.<sup>32</sup> Based on the data presented here, curcumin may prove to be a potential therapeutic agent for the prevention and possibly treatment of stenotic vascular remodeling and/or contributory mechanisms of atherosclerosis.

Curcumin has been used as a food additive in curry and in indigenous medicine for centuries. Nutritional supplementation with curcumin has also been credited with a variety of beneficial effects in chronic diseases.<sup>7,33</sup> Curcumin is among the National Institutes of Health list of drugs being evaluated clinically as a chemopreventive drug

in a variety of cancers.<sup>34</sup> Studies have consistently shown that curcumin is relatively nontoxic, even at doses much higher than those used in our current study.<sup>35</sup>

In summary, this is the first report to show that curcumin is capable of attenuating the neointimal response to experimental arterial injury, and, we believe, this occurs via attenuation of the PDGF signaling cascade. Aortic SMC migration, proliferation, and collagen synthesis, shown here to be inhibited by curcumin, are crucial events underlying the complications of vascular injury. Beside providing novel insights into the protective action of curcumin in the vascular injury response, these results offer a therapeutic potential of curcumin in the prevention and treatment of vascular diseases and restenosis after angioplasty.

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