Epothilone B Inhibits Neointimal Formation after Rat Carotid Injury through the Regulation of Cell Cycle-Related Proteins

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ABSTRACT

The abnormal proliferation of vascular smooth muscle cells (VSMCs) in arterial walls is an important pathogenetic factor of vascular disorders such as atherosclerosis and restenosis after angioplasty. Epothilone B, a novel potential antitumor compound, has a potent effect on preventing postangioplasty restenosis. Therefore, we established an in vivo rat carotid injury model and examined the potential effects of epothilone B on cardiovascular disease. We found that epothilone B potently prevented neointimal formation and in vivo VSMCs proliferation. In addition, we also showed that epothilone B significantly inhibited 5% fetal bovine serum (FBS)- and 50 ng/ml platelet-derived growth factor (PDGF)-BB-induced proliferation and cell cycle progression in rat aortic VSMCs. Furthermore, FBS and PDGF-BB induced the activations of extracellular signal-regulated kinases 1 and 2, Akt, phospholipase C γ 1, and PDGF-receptor β chain tyrosine kinase were not changed by epothilone B. However, epothilone B treatment caused a significant decrease in the level of cyclin-dependent protein kinase (CDK) 2, whereas it caused no change in the levels of cyclin E and down-regulated the phosphorylation of retinoblastoma, which plays a critical role in cell cycle regulation. Furthermore, levels of p27, an inhibitor of cyclin E/CDK2 complex, were significantly increased in VSMCs treated with epothilone B, indicating that this might be a major molecular mechanism for the inhibitory effects of epothilone B on the proliferation and cell cycle of VSMCs. These findings suggest that epothilone B can inhibit neointimal formation via the cell cycle arrest by the regulation of the cell cycle-related proteins in VSMCs.

Proliferation and migration of vascular smooth muscle cells (VSMCs) play a pivotal role in the development of restenosis and in the progression of atherosclerosis (Ross, 1993). Arterial injury results in the migration of VSMCs into the intimal layer of the arterial wall, where they proliferate and synthesize extracellular matrix components. Many growth factors induce the proliferation of VSMCs in vitro and in vivo. Among them, platelet-derived growth factor (PDGF) and basic fibroblast growth factor are important regulators of VSMC behavior through their well defined actions as potent chemoattractants and strong mitogens. Administration of these growth factors enhances intimal thickening after angioplasty in rat, whereas injection of antibodies or use of antisense technology to block signal transduction by either of these growth factors potently inhibits postinjury intimal hyperplasia in rat and restenosis in pig, suggesting that VSMC growth plays an important role in the pathogenesis of these models.

Arterial injury during percutaneous transluminal coronary angioplasty induces multiple signaling pathways that activate VSMC migration and proliferation. Immediately after injury, VSMCs leave their quiescent state and enter the cell...
cycle, which is associated with the induction of early-response genes (Harper et al., 1993; Serrano et al., 1993; Poljak et al., 1994). Cell division and growth are tightly controlled by a series of positive and negative regulators that act at sequential points throughout the cell cycle. The contribution of vascular proliferation to the pathophysiology of in-stent restenosis, transplant vasculopathy, and vein bypass graft failure is particularly important. Thus, an emerging strategy for the treatment of those conditions is to inhibit cell proliferation by targeting cell cycle regulation.

Cyclin-dependent protein kinases (CDKs) play central roles in the cell cycle in mammalian cells (Morgan, 1995). In addition, the existence of intrinsic CDK inhibitors has been demonstrated, which apparently bind to the CDK/cyclin complex and inhibit its kinase activity (Peters and Herskovitz, 1994). Recently, it was reported that antisense oligonucleotides targeted to CDK2 or gene transfer of p27 into a balloon-injured artery both led to inhibition of intimal thickening (Morishita et al., 1994; Chang et al., 1995; Chen et al., 1997).

Epothilone B is a novel potential antitumor compound that has been isolated from the myxobacterium Sorangium cellulosum. Epothilones show inhibitory effects on the cytoskeleton similar to those of taxol, an antineoplastic drug (Bollag et al., 1995), but unlike taxol, epothilone B-mediated microtubule stabilization does not trigger endotoxin signaling pathways (Muhlradt and Sasse, 1997). Although epothilone B can inhibit proliferation in multiple tumor cell lines (Altmann et al., 1994; Chang et al., 1995; Chen et al., 1997).

Preparation of Epothilone B-Containing Pluronic Gel. Thirty percent (w/v) F-127 Pluronic gel solution was prepared and kept at 4°C for 24 h. Two milligrams of epothilone B was dissolved in 1 ml of 100% ethanol, and 1 ml of this ethanolic solution was added to 9 ml of the previously cooled 30% Pluronic gel solution. A 100-μl aliquot of this solution thus contained 20 μg of epothilone B. Pluronic gel containing vehicle was formulated via the same method. In brief, the rats were anesthetized using an i.p. injection of ketamine (50 mg/kg) and xylazine (6.7 mg/kg), and the abdominal aorta of the rat was isolated by a midline abdominal incision. A 4F vascular cannula (Cook, Bloomington, IN) was introduced through the abdominal aorta and advanced to the thoracic aorta. Carotid cineangiography was performed by injecting contrast agent (Visipaque; Amersham Health, Cork, UK). Angiographic mean luminal diameters (MLDs) were measured using computerized coronary angiography (DCI Videodensitometry, Phillips, The Netherlands).

Morphometric Analysis. Immediately following the angiography, the rats were sacrificed, and morphometric analysis was performed. Carotid arteries were perfusion-fixed with 10% buffered formalin. Carotid artery sections (5 μm) were stained with hematoxylin-eosin, and morphometric analysis was performed using three individual sections from the middle of each Pluronic gel-applied arterial segment by an investigator blind to the experimental procedure. Cross-sectional areas (Antimia and Amedio), area ratios (Antimia/Amedio), and percent area stenosis were analyzed and calculated using Image Inside (version 2002; Focus, Daejeon, Korea).

Measurement of In Situ VSMC Proliferation. The effect of epothilone B on in situ VSMC proliferation was measured by BrdU incorporation on 2 days after injury as described previously (Sollott et al., 1995). In brief, epothilone B and vehicle-treated rats were injected s.c. with BrdU (30 mg/kg) at 30, 38, and 46 h after injury. The carotid artery sections were harvested at 48 h after injury, and histologic sections were incubated with mouse anti-BrdU mAbs (Roche Diagnostics, Indianapolis, IN). The fraction of BrdU-positive medial VSMC nuclei per cross-section was compared between epothilone B and vehicle groups.

Rat Aortic VSMCs Isolation and Culture. Rat aortic VSMCs were isolated by enzymatic dispersion as described previously (Kim et al., 2002). Cells were cultured in DMEM supplemented with 10% FBS, 100 U/ml penicillin, 100 μg/ml streptomycin, 8 mM HEPES, and 2 mM L-glutamine at 37°C in a humidified 95% air and 5% CO2 incubator. The purity of rat VSMCs in culture was confirmed by the Western blotting of α-smooth-muscle actin. VSMCs were used at passages 4 to 8.

In Vitro VSMC Proliferation Assay. The VSMCs were preincubated in serum-free medium in the presence or absence of epothilone B (0.1–100 nM) for 24 h and then stimulated with 5% FBS or 50 ng/ml PDGF-BB for 24 h. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was added to the medium, and the cells were incubated for 1 h. The optical density was determined at 560 nm using a microplate reader.

Cell Cycle Analysis. Rat aortic VSMCs in 60-mm2 dishes were incubated in DMEM without serum (Invitrogen), with/without epothilone B (0.1–100 nM) for 24 h. The cells were then treated with/without 5% FBS or 50 ng/ml PDGF-BB for 24 h and then were trypsinized and centrifuged at 1500g for 7 min. Centrifuged pellets were suspended in 1 ml of 1× phosphate-buffered saline, washed twice, and recentrifuged. Pellets were suspended in 70% ethanol and fixed overnight at 4°C. The fixed cells were briefly vortexed and
centrifuged at 15,000g for 5 min. Ethanol was discarded, and pellets were stained with 0.4 ml of propidium iodide (PI) solution (50 μg/ml PI in buffer containing 100 μg/ml RNase A). Before analysis by flow cytometry, samples were incubated for 1 h at room temperature. The complex of DNA-PI in cell nuclei was measured by FACSCalibur (BD Biosciences, San Jose, CA). Proportions of cells in the G0/G1, S, and G2/M phases were determined using the ModFitLT program (Verity House Soft-ware, Topsham, ME).

**Immunoblotting.** SDS-PAGE was performed on rat aortic VSMCs lysates using 10% acrylamide gels. Proteins were transferred to polyvinylidene difluoride membranes (Amersham Biosciences), and membranes were blocked overnight at 4°C in Tris-buffered saline containing 0.1% Tween 20 and then incubated with a 1:1000 dilution of anti-phospho-ERK1/2, anti-phospho-Akt, anti-phospho-PLCγ1, anti-ERK1/2, anti-Akt, anti-PLCγ1, anti-phospho-PDGFRβ, anti-PDGFRβ, anti-phospho-Rb, anti-CDK2, anti-cyclin E, anti-p27, and anti-α-actin antibodies. Blots were then washed with Tris-buffered saline containing 0.1% Tween 20 and incubated with a 1:5000 dilution of horseradish peroxidase-conjugated anti-mouse IgG or anti-rabbit IgG antibodies (New England Biolabs, Beverly, MA). The proteins were detected using an enhanced chemiluminescence Western blotting detection reagent (Amersham Biosciences). The intensities of the bands were quantified using the Scion Image for Windows program (Scion Corporation, Frederick, MD).

**Statistical Analysis.** The results are expressed as means ± S.D. Statistical comparisons between two groups were made using the two-tailed, unpaired Student’s t-test, whereas a one-way analysis of variance was also used for multiple comparisons, and this was followed by Dunnett’s test. These data were considered significant with a probability less than 0.05.

**Results**

**Epothilone B Inhibits Neointimal Formation after Rat Carotid Injury.** In the control angiogram, a distinct narrowing of the injured right carotid was evident (Fig. 1A). Luminal narrowing was markedly reduced in the epothilone B group (Fig. 1B). The MLD of the injured carotid artery was compared with the luminal diameter of the uninjured left carotid artery [set at 1 arbitrary unit (AU)]. The MLDs of the epothilone B-treated groups (0.81 ± 0.06 AU, n = 3) were significantly larger than that of the control (0.58 ± 0.02 AU, n = 3). On morphometric analysis, the epothilone B group (n = 10, 0.16 ± 0.03 mm²) showed a significant reduction in neointimal formation compared with the control (n = 8, 0.22 ± 0.05 mm²). The neointima/media area ratios (1.51 ± 0.37 versus 2.06 ± 0.46) and percent area stenosis (39.5 ± 13.2 versus 55.0 ± 13.3%) of epothilone B group was also reduced. However, the medial area (0.11 ± 0.02 versus 0.11 ± 0.01 mm²) was unchanged (Fig. 2A; Table 1). Pluronic gel in this study was applied only to the quadrant of the

![Image](50x132 to 290x348)

Fig. 1. The representative carotid angiographic findings of the control (A) and epothilone B-treated group (B) performed 14 days after rat carotid artery injury. Diffuse and significant narrowing of the right carotid artery was observed in the control group. The epothilone B group showed significant reduction in the luminal narrowing of carotid artery compared with the control group (A, MLD, 0.58 ± 0.02 AU, n = 3; B, MLD, 0.81 ± 0.06 AU, n = 3). Results are expressed as mean ± S.D. * P < 0.05 versus control.

![Image](300x260 to 557x556)

Fig. 2. A, representative cross-sections of rat carotid arteries taken 14 days after balloon injury and in vivo medial VSMC proliferation. Epothilone B inhibited the accumulation of intimal smooth muscle cells 14 days after balloon catheter injury of rat carotid artery. Note the eccentric pattern of inhibition resulting from application of Pluronic gel over roughly one fourth to one third of the circumference of the vessel wall. a, control; b, epothilone B. Magnification, ×100 and ×400. B, epothilone B inhibited in vivo medial VSMC proliferation. In situ VSMC proliferation was assessed by BrdU labeling 2 days after injury. a, control; b, epothilone B.

**TABLE 1**

Histomorphometric indices of epothilone B group

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<th>Control (n = 8)</th>
<th>Epothilone B (n = 10)</th>
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<tr>
<td>Neointimal area (mm²)</td>
<td>0.22 ± 0.05</td>
<td>0.16 ± 0.03*</td>
</tr>
<tr>
<td>Neointima/media area ratio</td>
<td>2.06 ± 0.46</td>
<td>1.51 ± 0.37*</td>
</tr>
<tr>
<td>% Area stenosis</td>
<td>55.0 ± 13.3</td>
<td>39.5 ± 13.2*</td>
</tr>
<tr>
<td>Media area (mm²)</td>
<td>0.11 ± 0.01</td>
<td>0.11 ± 0.02</td>
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* P < 0.05 vs. control.
vessel exposed during the surgery. Comparison of the neointimal area of the entire vessel results in a significant underestimation of the degree of neointimal inhibition. Therefore, we also analyzed the neointima/media area ratios of drug-applied and nonapplied quadrants. As expected, the neointima/media area ratios showed a significant difference between the drug-applied and nonapplied quadrants (Table 2). All Pluronic gel-applied sections were examined grossly and histologically for evidence of inflammation. Fourteen days after the Pluronic gel was applied, they were completely degraded, leaving no materials behind. At the time of sacrifice, there was no gross evidence of an inflammatory or foreign body response to either the drug- or vehicle-loaded Pluronic gel. This was confirmed by histological examination of the injured vascular tissue, which was devoid of signs of inflammation in the adventitia and media of treated vessels (data not shown).

**Epothilone B Inhibits in Vivo Medial VSMC Proliferation.** The effect of epothilone B on in situ VSMC proliferation was measured by BrdU incorporation 2 days after injury. In vivo medial VSMC proliferation was significantly inhibited in the epothilone B-treated group at 2 days after injury (control versus epothilone B, 25.5 ± 4.8 versus 16.6 ± 1.2%, n = 3, per group, P < 0.05) (Fig. 2B).

**Epothilone B Inhibits VSMC Proliferation and Cell Cycle Progression in Vitro.** Next, we tested the hypothesis that its use would block the growth of VSMCs. Therefore, the effect of epothilone B on in vitro VSMC proliferation was measured by MTT assay. Compared with untreated cells, pretreatment with epothilone B (0.1–100 nM) significantly decreased in vitro VSMC proliferation in a concentration-dependent manner (Fig. 3A, inhibition percentages of FBS-stimulated VSMC proliferation, 45, 63, 90, and 91%; Fig. 3B, inhibition percentages of PDGF-BB-stimulated VSMC proliferation, 30, 47, 88, and 91%, at the concentrations of 0.1, 1, 10, and 100 nM, respectively). To test the generality of the effects of epothilone B on cell cycle, we examined its effect on

![Fig. 3](https://example.com/fig3.png)

**Fig. 3.** Effects of epothilone B on FBS- and PDGF-BB-stimulated VSMC proliferation in vitro. The VSMCs were precultured in the presence or absence of epothilone B (0.1–100 nM) in serum-depleted medium for 24 h, and then VSMCs were stimulated by 5% FBS (A) or 50 ng/ml PDGF-BB (B). After 24 h, MTT was added to the medium, and the cells were incubated for 1 h. The optical density was determined at 560 nm using a microplate reader. Each value represents the mean ± S.D. from three different sets of assay. *, P < 0.05; **, P < 0.01 compared with FBS or PDGF-BB alone.

![Fig. 4](https://example.com/fig4.png)

**Fig. 4.** Effects of epothilone B on FBS- and PDGF-BB-stimulated cell cycle progression. The VSMCs were precultured in the presence or absence of epothilone B (0.1–100 nM) in serum-depleted medium for 24 h, and then VSMCs were stimulated by 5% FBS (A) or 50 ng/ml PDGF-BB (B). After 24 h, individual nuclear DNA content was reflected by fluorescence intensity of incorporated propidium iodide. Each item is derived from a representative experiment, where data from at least 10,000 events were obtained. Data are representative of at least three independent experiments with similar results.

**TABLE 2**

Neointima/media area ratios of Pluronic gel-applied quadrants and non-applied quadrants

<table>
<thead>
<tr>
<th>Pluronic Gel</th>
<th>Applied Quadrant</th>
<th>Nonapplied Quadrant</th>
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<tr>
<td>Control (n = 8)</td>
<td>2.06 ± 0.56</td>
<td>2.10 ± 0.30</td>
</tr>
<tr>
<td>Epothilone B (n = 10)</td>
<td>1.20 ± 0.36*†</td>
<td>1.83 ± 0.51</td>
</tr>
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* P < 0.05 vs. control, among the applied quadrants.
† P < 0.05, between the applied quadrant and non-applied quadrant.
cell cycle progression elicited by FBS (5%) or PDGF-BB (50 ng/ml). Epothilone B (0.1–100 nM) potently blocked both FBS- and PDGF-BB-induced cell cycle progressions in a concentration-dependent manner (Fig. 4). We also performed cell counts and [3H]thymidine incorporation assay to confirm that the effect of epothilone B on cell cycle progression in VSMCs truly reflected changes in proliferation, and similar results were obtained in both cell counting and [3H]thymidine incorporation assay experiments (data not shown).

**Effects of Epothilone B on ERK1/2, Akt, PLCγ1, and PDGF-Rβ Activations.** By immunoblotting analysis, we measured ERK1/2, Akt, and PLCγ1 activities as a number of critical growth signaling pathways. We have used U0126, LY294002, and U73122 as selective inhibitors for ERK1/2, Akt, and PLCγ1 activation. Increased amounts of phosphorylated ERK1/2, Akt, and PLCγ1, compared with untreated cells, were detected after stimulation of VSMCs with FBS (5%) or PDGF-BB (50 ng/ml) for 5 to 15 min. Phosphorylations of ERK1/2, Akt, and PLCγ1 stimulated by FBS and PDGF-BB were blocked by pretreatment with U0126, LY294002, and U73122 but not with epothilone B (Fig. 5, A–C). We also addressed whether epothilone B influenced PDGF-BB-induced activation of PDGF-Rβ tyrosine kinase. We used tyrphostin AG1295 as a selective inhibitor for PDGF-Rβ tyrosine kinase. Increased amounts of phosphorylated PDGF-Rβ tyrosine kinase compared with untreated cells were detected after stimulation of VSMCs with PDGF-BB (50 ng/ml) for 1 min. Phosphorylation of PDGF-Rβ tyrosine kinase by PDGF-BB was blocked by pretreatment with tyrphostin AG1295 but not with epothilone B (Fig. 5D). These results provide evidence that the effect of epothilone B on VSMC proliferation may be due to a specific arrest of the cell-cycle machinery without affecting upstream mitogenic signaling events.

**Epothilone B Inhibits CDK2 Level and Rb Phosphorylation through p27 Protein Increase.** To examine the specific effect of epothilone B on the VSMC proliferation and cell cycle machinery, we assessed cell cycle-related proteins in cellular lysates from PDGF-BB-stimulated VSMCs. To investigate the mechanism of epothilone B-induced cell cycle checkpoint, VSMCs treated with epothilone B (100 nM) from 6 to 24 h were subjected to Western blot analysis (Fig. 6A). Among several cell cycle-related proteins, CDK2 and cyclin E protein levels were up-regulated in response to PDGF-BB stimulation, whereas the increased CDK2 was significantly blocked, but cyclin E level was not altered by pretreatment with epothilone B. The level of p27 protein, a CDK inhibitor, was also increased by the treatment of epothilone B (0.1–100 nM) in a concentration-dependent manner. These results indicate that the epothilone B-induced cell cycle arrest can be associated with the CDK2 protein at the G0/G1 phase. The phosphorylation of Rb protein mediated by cyclin E/CDK2 is necessary for cells to progress from G0/G1 to S phase. As a final measurement of cell cycle-related proteins, the effect of epothilone B on Rb phosphorylation was examined. The result showed that PDGF-BB-induced phosphorylation of Rb...
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Figure 6. Effects of epothilone B on cell cycle-related proteins in PDGF-BB-stimulated VSMCs. A, VSMCs were cultured in 12-well plates until confluence, and the medium was replaced with serum-free medium in the presence or absence of epothilone B (100 nM) for 24 h. Then, the VSMCs were stimulated with 50 ng/ml PDGF-BB for 6 to 24 h. B, VSMCs were cultured in 12-well plates until confluence, and the medium was replaced with serum-free medium in the presence or absence of epothilone B (0.1–100 nM) for 24 h. The cells were lysed, and proteins were subjected to 10% SDS-PAGE. Similar results were obtained in three independent experiments. Quantitation of bands was done by densitometric analysis and is shown as fold of basal compared with vehicle control at the bottom of the bands.

was significantly decreased by the treatment of epothilone B in a concentration-dependent manner (Fig. 6). Taken together, these results indicate that epothilone B influences the activity of G0/G1 and S phase-related cell cycle proteins in association with its antiproliferative effects in rat aortic VSMCs.

Discussion

Cardiovascular diseases, which are a leading cause of mortality and morbidity in the Western world, are associated with a multitude of pathophysiologic conditions, such as inflammation, pulmonary hypertension, coronary artery restenosis following balloon angioplasty, and VSMC proliferation in response to vessel injury (Ross, 1993). Therefore, an understanding of its inhibition on VSMC proliferation is important in terms of developing methods of treating cardiovascular disease.

In the present study, the rat carotid artery injury model was used to characterize the effect of epothilone B on the vascular response to injury. The polymer used in this study for local delivery of epothilone B was F-127 Pluronic gel as described previously (Wu et al., 2005). This polymer has a novel property of being soluble at 4°C, while solidifying on contact with tissues at 37°C. This characteristic makes it useful in pharmaceutical compounding because it can be drawn into a syringe for accurate dose measurement when it is cold. Pluronic gel was applied directly to the exposed adventitial surface over the site of injury with the effect on neointimal growth as shown in Fig. 2A, a. The molar concentration of epothilone B was 0.394 mM in 100 μl of Pluronic gel containing epothilone B, and local application of epothilone B produced a highly eccentric pattern of inhibition of neointimal growth in the arterial cross-section (Fig. 2A, b). In the current study, there was no gross evidence of an inflammatory or foreign body in response to either the drug- or vehicle-loaded Pluronic gel. This eccentric response was correlated with the site of Pluronic gel application, which was limited to the surface of the vessel wall exposed during the surgical procedure (approximately 25–40%). As expected, the control Pluronic gel had no effect, yielding a robust concentric neointimal growth (Fig. 2A; Table 2). The eccentric response to epothilone B demonstrates its focal effect on neointimal growth at the site of application. The ratio of the neointimal to medial wall areas showed a significant difference between the drug-applied and nonapplied areas. Thus, the inhibition was only detected in that portion of the vessel in direct contact with the epothilone B-containing Pluronic gel or in an immediately adjacent region, indicating the site-specific, localized action of the drug. From the tissue sensitivity to epothilone B and its pattern of inhibition, we would expect that exposure of the entire adventitial surface to epothilone B would lead to the profound inhibition of neointimal development in the injured vessel (Signore et al., 2001). Although the perivascular administration of epothilone B is not a technically feasible approach for treating coronary restenosis after percutaneous coronary intervention in humans, it may have application in other surgical procedures in which stenosis represents a significant complication. Procedures in which the extravascular surface is accessible for application of the drug include coronary artery bypass surgery and the creation of graft anastomosis in hemodialysis patients with arteriovenous shunts. Epothilone B could also be a candidate agent for drug-eluting stents (Heldman et al., 2001; Park et al., 2003).

During atherosclerotic lesion progression, VSMC proliferation is of particular pathophysiologic importance (Gordon et al., 1990). In atherosclerotic lesions, VSMCs are exposed to mitogenic substances such as PDGF (Cui et al., 1998; Gutstein et al., 1999). Moreover, the association between PDGF and VSMC proliferation has been demonstrated in animal experiments, in which increases and augmentations of PDGF-BB after arterial injury were found to be correlated with neointimal cellular proliferation (Uchida et al., 1996). In the present study, we investigated the inhibitory effect of epothilone B on FBS- and PDGF-BB-induced VSMC proliferation and a possible sig-
Epothilone B was found to inhibit VSMC proliferation in vitro (Fig. 3) and DNA synthesis (data not shown) induced by FBS and PDGF-BB in a concentration-dependent manner, and these results agreed with those of other studies, which showed that taxol, a well-known drug for cardiovascular disease, inhibits VSMC proliferation (Blagosklonny et al., 2006; Speck et al., 2006). Moreover, the present study showed a concentration-dependent G0/G1 arrest in VSMCs following treatment with epothilone B (Fig. 4). Next, we aimed to elucidate the molecular mechanism of epothilone B on VSMC proliferation. PDGF-induced mitogenesis and proliferation are also known to be prerequisites for the intimal thickening that is observed after angioplasty (Sachinidis et al., 1990). PDGF-BB binding to PDGF-Rβ leads to its phosphorylation at multiple tyrosine residues. This activated PDGF-Rβ is associated with signaling pathways, such as ERK1/2, Akt, and PLCγ1 (Mulvany, 1990; Claesson-Welsh, 1994; Heldin et al., 1998). Moreover, PDGF-Rβ antagonist or protein kinase inhibitor reduced VSMC proliferation in vitro and prevented cardiovascular disorders in several animal experiments (Waltenberger, 1997; Lipson et al., 1998; Mylläriemi et al., 1999). In this study, we found that the inhibitory effects of epothilone B on cell cycle progression and proliferation of VSMC were not mediated via the suppressions of the ERK1/2, Akt, and PLCγ1 signaling pathways (Fig. 5). Moreover, we observed that PDGF-Rβ phosphorylation was not altered by epothilone B at 0.1 to 100 nM (Fig. 5D). Thus, we thought that epothilone B might directly target some species of the cell cycle-regulated proteins to inhibit VSMC proliferation and cell cycle.

Sriram and Patterson (2001) have demonstrated that a key regulator of the G0/G1 to S phase transition in the cell cycle is Rb, a tumor suppressor protein. The present study demonstrated that epothilone B down-regulated the phosphorylation of Rb (Fig. 6). In addition, epothilone B treatment caused a significant decrease in the level of CDK2 complex but no change in the level of cyclin E. Furthermore, p27, an inhibitor of cyclin E/CDK2 complex, plays a critical role in cell cycle regulation by binding and inhibiting various CDK/cyclin complex activities (Sherr, 1994; Morgan, 1995). The level of p27 was increased in cells treated with epothilone B (Fig. 6B), indicating that this might be a major molecular mechanism for the inhibitory effects of epothilone B on the growth and cell cycle of VSMCs.

In summary, we have demonstrated that epothilone B induces cell cycle arrest, inhibits VSMC proliferation in vitro, and reduces neointimal formation in vivo after local epothilone B treatment. Our results indicate that epothilone B can inhibit VSMC proliferation and neointimal formation via the cell cycle arrest by the regulation of the cell cycle-related proteins in VSMC (Fig. 7).

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References


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