TAS-301 Blocks Receptor-Operated Calcium Influx and Inhibits Rat Vascular Smooth Muscle Cell Proliferation Induced by Basic Fibroblast Growth Factor and Platelet-Derived Growth Factor

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Received March 31, 2000   Accepted July 24, 2000

ABSTRACT—The purpose of this study was to determine the effect of a recently synthesized drug, TAS-301 [3-bis(4-methoxyphenyl)methylenylene-2-indololone], on vascular smooth muscle cell (VSMC) proliferation and the intracellular signal transduction pathways involved in VSMC proliferation. In an in vitro assay, TAS-301 inhibited the proliferation of rat VSMCs stimulated by platelet-derived growth factor (PDGF)-BB, basic fibroblast growth factor, or 2% fetal bovine serum in a concentration-dependent manner. TAS-301 dose-dependently inhibited the PDGF-induced Ca²⁺ influx; the concentration for the inhibition of Ca²⁺ influx was nearly identical to that for inhibition of VSMC proliferation. The Ca²⁺ influx induced by PDGF was also attenuated by NiCl₂ but not by nifedipine, suggesting that PDGF-induced Ca²⁺ influx would be mediated by some non-voltage-dependent mechanisms. Furthermore, TAS-301 inhibited PDGF-induced activation of protein kinase C (PKC) and the phorbol 12-myristate 13-acetate-mediated induction of activator protein 1 (AP-1) in a concentration-dependent manner. These findings indicate that TAS-301 inhibited the proliferation of VSMCs by blocking voltage-independent Ca²⁺ influx and downstream signals such as the Ca²⁺/PKC signaling pathway, leading to AP-1 induction.

Keywords: TAS-301, Vascular smooth muscle cell proliferation, Receptor-operated calcium influx, Signal transduction

Abnormal vascular smooth muscle cell (VSMC) proliferation is a key process underlying the formation of atherosclerotic plaques (1). It is also a pathological feature of restenosis that limits the efficacy of percutaneous transluminal coronary angioplasty (PTCA) (2). There is, therefore, considerable interest in inhibitors of VSMC proliferation. From experiments using specific antibodies, growth factors such as basic fibroblast growth factor (bFGF) and platelet-derived growth factor (PDGF) have been shown to have a pivotal role in VSMC proliferation and to control specific events in the neointimal formation after balloon injury (3, 4). Therefore, for preventing restenosis after PTCA, one approach is to develop inhibitors that interfere with the common mitogenic signals activated by these growth factors. Growth factors such as bFGF and PDGF trigger a number of signaling pathways to initiate proliferation, including activation of receptor-associated tyrosine kinases, phospholipases, and other tyrosine or serine/threonine kinases (5, 6). Among these signaling molecules, mitogen-activated protein kinases (MAPKs), known as extracellular signal-regulated kinases (ERKs), are a family of protein-serine/threonine kinases, and in their activated forms, p44 MAPK (ERK1) and p42 MAPK (ERK2), are believed to play a central role in cellular proliferation (7). Likewise, protein kinase C (PKC), activated by diacylglycerol or phorbol esters such as phorbol 12-myristate 13-acetate (PMA), also appears to play a prominent role in initiating cell proliferation (8). ERK and PKC-mediated signal pathways finally lead to the induction of the transcription factor complex activator protein 1 (AP-1) (9, 10). AP-1 complexes are composed of members of the Jun and Fos
families and bind to specific control elements present in the promoters of genes that regulate cellular proliferation (9, 10).

Tyrosine phosphorylation of growth factor receptors induces an increase in the intracellular Ca^{2+} concentration ([Ca^{2+}]_i) by causing Ca^{2+} to be released from intracellular stores and by causing Ca^{2+} influx from the extracellular space. The release of intracellular Ca^{2+} is most likely mediated by inositol 1,4,5-trisphosphate, but the mechanism of Ca^{2+} influx is still not fully understood (11). Recent studies indicated that the Ca^{2+} mobilization from intracellular stores may not be a critical event in DNA synthesis induced by PDGF (12, 13); on the other hand, the Ca^{2+} influx may be prerequisite for PDGF-induced DNA synthesis (14, 15). Therefore, the influx of Ca^{2+} from the extracellular space after stimulation by growth factors may be important in VSMC proliferation.

In our previous report, we showed that the new drug TAS-301 [3-bis(4-methoxyphenyl)methylene-2-indolinone] (Fig. 1) inhibited neointimal thickening after balloon catheter injury to the rat common carotid artery, and this compound reduced the proliferation of medial and intimal VSMCs after the injury (16). Based on the results of the present study, we report that TAS-301 displays potent inhibitory effects on the proliferation process of cultured rat VSMCs; and we propose a possible molecular mechanism by which TAS-301 inhibits VSMC proliferation.

MATERIALS AND METHODS

Materials

We used TAS-301 (Fig. 1), synthesized by Taiho Pharmaceuticals (Hanno City) and the following reagents (source in brackets): Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), ITS (5 μg/ml insulin, 5 μg/ml transferrin and 5 ng/ml selenium), and PDGF-BB [Life Technologies Inc., Grand Island, NY, USA]; [γ-32P]ATP, [35S]ATP and [Ca^{2+}]_i (Amersham, Arlington Heights, IL, USA); bovine serum albumin (BSA) and phorbol 12-myristate 13-acetate (PMA) (Sigma Chemical Co., St. Louis, MO, USA); PD98059 (2-[amino-3-methoxyphenyl]-4H-1-benzopyran-4-one) (Calbiochem, La Jolla, CA, USA); GF109203X (3-[3-(dimethylamino)propyl]-1H-indol-3-yl)-4-(1H-indol-3-yl)-1H-pyrrrole-2,5-dione (RBI, Natick, MA, USA); dexamethasone (Nacalai Tesque, Kyoto); pGL2-basic promoter vector and reporter lysis buffer (Promega, Madison, WI, USA). Other chemicals were of the highest grade commercially available.

Cell culture

VSMCs were prepared from the thoracic aortic media of 12- to 13-week-old Sprague-Dawley rats (Clea Japan, Tokyo) by the explant method and were cultured in DMEM containing 10% FBS, 2 mM l-glutamine, 100 units/ml penicillin and 100 μg/ml streptomycin, in a humidified 5% CO_2 atmosphere at 37°C as described previously (17). Subcultured VSMC from passages 3–11 were used in the experiments, and they showed >98% positive immunostaining with a smooth muscle actin antibody (clone 1A4; Dako, Copenhagen, Denmark) and were negative for mycoplasmas. For the experiments, cells at approximately 80% confluence in culture wells were made quiescent by incubation with serum-free DMEM containing 0.1% BSA supplemented with or without ITS for 2–3 days, unless otherwise stated.

All test drugs were dissolved in dimethylsulfoxide (DMSO) and added to the culture medium in appropriate concentrations. Control groups were cultured with an equivalent amount of solvent without the drug. TAS-301 was applied 2 h before cells were stimulated, and other specific inhibitors were added 30 min before stimulation, unless otherwise stated.

In vitro proliferation assay

Cell proliferation was determined by the incorporation of 5-bromo-2′-deoxyuridine (BrdU) by quiescent cells as described previously (18). In brief, cells were seeded at a density of 1 × 10^4 cells per well into 96-well plates in DMEM containing 10% FBS. Two days after the seeding, the growth was arrested for 3 days in serum-free DMEM containing ITS. The DMEM/ITS was then removed, and serum-free DMEM containing 0.1% BSA with or without TAS-301 was added to the quiescent cells 2 h before treatment with PDGF-BB (10 ng/ml), bFGF (0.1 ng/ml) or 2% FBS. At 24 h after stimulation, BrdU (10 μM) was added to the cells; 24 h later, the cells were fixed. An ELISA was used according to the supplier’s recommendations (Amersham, Buckinghamshire, England) to quantify the incorporated BrdU. TAS-301 was present during the entire experi-
Measurement of ERK activity

ERK assays were performed as described previously (19). Briefly, cells grown in 24-well plates were arrested for 3 days in serum-free DMEM/ITS and then stimulated with PDGF-BB. The reaction was terminated by the replacement of the medium with ice-cold lysis buffer (10 mM Tris-HCl, 20 mM NaCl, 2 mM EGTA, 2 mM DTT, 1 mM Na3VO4, 1 mM phenylmethylsulfonyl fluoride (PMSF), 10 μg/ml leupeptin, 10 μg/ml aprotinin, pH 7.4). After brief sonication, the samples were centrifuged (14,000 × g, 5 min), and the supernatant was assayed for ERK activity with an assay kit (Amersham Corp.) that measures the incorporation of [γ-32P]ATP into a synthetic peptide designed as a specific ERK substrate. The radioactivity was measured by a liquid scintillation counter (2000CA; Packard, Meriden, CT, USA). The specific activity of each sample was calculated after subtraction of basal activity and was expressed as picomoles of 32P transferred to the specific substrate/min/mg protein.

Measurement of PKC activity

Serum-starved cells grown in 6-well plates (serum-free DMEM/ITS for 3 days) were stimulated with PDGF-BB. The cells were then separated into cytosolic and particulate fractions, and the PKC activity of the particulate fraction was measured by a modified version of a previously reported method (20). Briefly, the stimulation with PDGF-BB was terminated by the replacement of the medium with ice-cold lysis buffer (50 mM Tris-HCl, 5 mM EGTA, 10 mM EDTA, 10 mM benzamidine, 0.3% 2-mercaptoethanol, 50 μg/ml PMSF, 10 μg/ml leupeptin, 10 μg/ml aprotinin, pH 7.4). The cells were then scraped with a rubber policeman and disrupted by sonication and centrifuged at 45,000 × g for 30 min. The pellet was resuspended in lysis buffer containing 1% nonidet-P40 (NP-40), sonicated and kept at 4°C for 60 min. PKC activity was measured with the Amersham PKC assay system. In this system, PKC catalyzed the transfer of [γ-32P]ATP to a peptide substrate specific for PKC. The radioactivity was measured by a liquid scintillation counter (2000CA). The specific activity of each sample was calculated after subtraction of basal activity and was expressed as picomoles of 32P transferred to the specific substrate/min·mg protein·mg−1 of membrane protein.

Luciferase assay (construction of luciferase plasmids and transfection)

PCR amplification products of −73/63 human collagenase gene promoter were cloned into a pGGL2-basic promoter vector upstream of the luciferase gene. In addition, oligonucleotides corresponding to 2 × 12-O-tetradecanoylphorbol-13-acetate-response elements (TRE) were synthesized, annealed and inserted into the upstream of the collagenase promoter to generate 3 × TREpGL2COL.

VSMCs were seeded in 6-well plates and transiently transfected with SuperFect transfection reagent according to the supplier’s recommendations (Qiagen, Inc., Valencia, CA, USA). To accomplish this, construct 3 × TREpGL2COL (2 mg/well) was diluted with serum-free DMEM and mixed with SuperFect transfection reagent to form a transfection complex. Then, DMEM with 0.5% FBS was added to the reaction tube containing the transfection complexes, and the total volume was then immediately transferred to the cells in the well. After a 4-h incubation with the transfection complex, the cells were washed and the medium was changed to serum-free DMEM. After incubation for 22 h, test compounds were added to the well; and 2 h later, the cells were stimulated with 30 ng/ml PMA for 5 h, harvested in ice-cold PBS, and lysed in lysis buffer following the manufacturer’s instructions. Luciferase activity was measured with a Luminescencer (AB-2000; Atto, Tokyo) and expressed as the fold increase above the value for unstimulated cells.

Measurement of unidirectional Ca2+ influx rate

Unidirectional Ca2+ influx rate was determined by measuring the initial uptake of [45Ca]. VSMCs were grown to confluence in 6-well plates and were serum-starved as described above. They were first pretreated with the desired inhibitor, then stimulated with PDGF-BB, and subsequently treated with 74 kBq/ml [45Ca]CaCl2 for 5 min. The cells were next washed with ice-cold HEPES buffer (120 mM NaCl, 5 mM CaCl2, 20 mM HEPES/NaOH, pH 7.8), solubilized by adding 0.1% SDS, and measured for their [45Ca] content by a liquid scintillation counter. Ca2+ influx rate (%) was calculated by measuring the amount of [45Ca] taken up during a 5-min period (nmol·mg protein−1·5 min−1).

Statistical analyses

Data were expressed as the means ± S.E.M. of four or six separate experiments. Multiple comparisons with controls (vehicle) were performed by Dunnett’s multiple comparison test on a JMP ver 3.0.2 software program (SAS Institute, Cary, NC, USA). Differences with a P<0.05 were considered to be statistically significant.

RESULTS

Proliferation of VSMCs

Pronounced BrdU incorporation by the cells, an index of DNA synthesis, was induced by the treatment with PDGF-BB (10 ng/ml), bFGF (0.1 ng/ml) or 2% FBS. The effects of TAS-301 on BrdU incorporation induced by these growth factors or serum are shown in Table 1. TAS-301
reduced the BrdU incorporation induced by all of the stimulators tested in a concentration-dependent manner (1–10 μM) and with significant inhibition at 10 μM (percent inhibition: 53.3%, 72.2% and 17.6% for PDGF-BB, bFGF- and serum-induced BrdU incorporation, respectively). In particular, TAS-301 showed a high potency for blocking bFGF-stimulated incorporation and significantly inhibited it at the concentration of 3 μM. This inhibition was not due to nonspecific cytotoxicity because the WST assay and LDH assay of cell supernatants demonstrated that TAS-301 (1–10 μM) exerted no significant effects on the viability of the cells (data not shown).

**PDGF-induced ERK activation**

The activity of ERK was augmented and peaked at 5 min after stimulation with PDGF, and then it declined slowly. The elevation of this kinase activity depended on the PDGF concentration (data not shown). Treatment with 10 ng/ml PDGF increased the activity to 34.8-fold above the level seen in unstimulated cells. Subsequent studies were therefore performed using 10 ng/ml PDGF for 5 min. The effects of TAS-301 on PDGF-induced ERK activation are shown in Fig. 2. TAS-301 (1–10 μM) did not affect the elevation of ERK activity induced by PDGF. On the other hand, PD 98059 (30 μM), an inhibitor of MAPK kinase (MEK), which activates ERK1 and ERK2, significantly inhibited PDGF-induced activation of ERK by 67.0%.

**PDGF-induced PKC activation**

Treatment of the VSMCs with 30 ng/ml PDGF caused an increase in the activity of PKC by 2.8-fold above the basal level at 5 min after PDGF addition, and the activity then declined slowly. The elevation of this kinase activity depended on the PDGF-BB concentration (data not shown). Therefore, subsequent experiments were performed using 30 ng/ml PDGF for 5 min. The effects of TAS-301 on PDGF-induced PKC activation are shown in Fig. 3. TAS-301 (1–10 μM) showed a concentration-dependent inhibition of PKC activation induced by PDGF, with a significant inhibition at 10 μM of 62.7%. GF109203X (1 μM), a spec-

<table>
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<th>Compounds</th>
<th>Conc. (μM)</th>
<th>PDGF-BB</th>
<th>bFGF</th>
<th>2% FBS</th>
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<td></td>
<td>O.D.</td>
<td>% inhibition</td>
<td>O.D.</td>
<td>% inhibition</td>
</tr>
<tr>
<td>Vehicle</td>
<td>0.995 ± 0.075</td>
<td>-8.1</td>
<td>1.371 ± 0.105</td>
<td>6.6</td>
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<td>TAS-301</td>
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<td>18.0</td>
<td>1.280 ± 0.0121</td>
<td>42.9</td>
</tr>
<tr>
<td></td>
<td>0.816 ± 0.091</td>
<td>53.3</td>
<td>0.783 ± 0.148**</td>
<td>72.2</td>
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<tr>
<td></td>
<td>0.465 ± 0.109**</td>
<td>**</td>
<td>0.381 ± 0.125**</td>
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The values are each a mean ± S.E.M. Data show BrdU incorporation (change in O.D.) induced by PDGF-BB (10 ng/ml, n = 7), bFGF (0.1 ng/ml, n = 6) and FBS (2%, n = 6). Statistical analysis was performed by Dunnett’s test. **P<0.01 compared with the vehicle control.

![Fig. 2. Effect of TAS-301 on PDGF-induced ERK activation. Quiescent rat VSMCs were pretreated with PD98059 (30 μM) for 30 min or TAS-301 (1, 3, 10 μM) for 2 h and then stimulated with 30 ng/ml PDGF-BB for 5 min. The cells were then lysed, and ERK activity was measured with the assay system described in Materials and Methods. Values shown are the means ± S.E.M. of six experiments. **P<0.01, significantly different from PDGF-treated cells (Dunnett’s test).](image)

![Fig. 3. Effect of TAS-301 on PDGF-induced PKC activation. Quiescent rat VSMCs were pretreated with GF109203X (1 μM) for 30 min or TAS-301 (1, 3, 10 μM) for 2 h and then stimulated with 30 ng/ml PDGF-BB for 5 min. Cells were then disrupted by sonication. Cytosolic and particulate fractions were separated, and PKC activity of the particulate fraction was measured with the assay system described in Materials and Methods. Values are shown as the means ± S.E.M. of five experiments. **P<0.01, significantly different from PDGF-treated cells (Dunnett’s test).](image)
cific inhibitor of PKC, also significantly inhibited the
PDGF-induced activation of PKC, by 92.1%.

**PMA-mediated AP-1 induction**

The luciferase activity driven by AP-1 was 2836 RLU
(relative luciferase unit) in the unstimulated cells. Treatment with 30 ng/ml PMA increased the AP-1 level to 3.3-fold above that for the unstimulated cells. The effects of TAS-301 on this AP-1 induction are shown in Fig. 4. The treatment with TAS-301 (1–10 μM) reduced the PMA-stimulated induction of AP-1 in a concentration-dependent manner, and significantly inhibited it at concentrations of 3 and 10 μM, by 38.0% and 67.6%, respectively. Under the same conditions, dexamethasone (0.1 μM) also caused a significant reduction in the PMA-mediated AP-1 induction, by 41.0%.

**PDGF-induced Ca²⁺ influx**

The Ca²⁺ influx rate increased at 5 min after the addition of 3 ng/ml PDGF-BB and increased gradually thereafter. After a 10-min stimulation, the Ca²⁺ influx reached a plateau, with an increase to a rate approximately 150% of that for the unstimulated cells (data not shown). Fig. 5A demonstrates the effect of TAS-301 on the Ca²⁺ influx induced by 3 ng/ml PDGF over a 10-min stimulation period. At concentrations higher than 3 μM, TAS-301 significantly inhibited the PDGF-induced Ca²⁺ influx. In the same type of experiment, PDGF-induced Ca²⁺ influx was not affected by 1 μM nifedipine, a blocker of L-type voltage-dependent

Ca²⁺ channels, whereas it was significantly inhibited by 5 mM NiCl₂ (Fig. 5B).

**DISCUSSION**

The present results demonstrated that TAS-301 inhibited growth factor-induced VSMC proliferation by modulating the mitogenic signal transduction pathways.

The proliferation of VSMCs is thought to contribute to atherosclerosis and restenosis after arterial injury, and several growth factors are thought to be involved in this event (21). Therefore, considerable efforts have been made to find effective drugs aimed at inhibiting growth factor-stimulated VSMC proliferation. However, given the multi-
plicity of the growth factors involved in VSMC proliferation, drugs targeted at a single growth factor may be less than optimally effective therapeutic agents. An alternative approach is to modulate the intracellular signaling cascade that is shared by many growth factors. Among growth factors involved in VSMC proliferation, PDGF-BB and bFGF are considered to be a potent mitogen of VSMCs and were shown to play a key role in the regulation of VSMC proliferation after balloon injury (22). These growth factors show substantial overlap in their signal transduction mechanisms, for example, stimulation of receptor tyrosine kinases followed by activation of mitogen-activated protein kinase (ERK1 and ERK2) and PKC and triggering of Ca\(^{2+}\) mobilization, which may lead to mitogenic responses in VSMCs (23–25). Therefore, the inhibition of these mitogenic signal molecules would be of great benefit for inhibiting VSMC proliferation stimulated by multiple growth factors.

In our in vitro study using cultured rat VSMCs, cells were stimulated by PDGF-BB, by bFGF, and by serum, a complex mitogen. These growth factors induced the proliferation of VSMCs as demonstrated by BrdU-incorporation measurements, and TAS-301 showed a potent and dose-dependent inhibitory effect on the all of BrdU incorporation induced by these growth factors. This strongly indicates that TAS-301 does not inhibit the binding of these growth factors to its receptor but acts downstream of receptor activation to exert its anti-proliferative action. In this study, we investigated the effect of TAS-301 on the post-receptor signal transduction pathway by using PDGF-BB as the stimulator.

We first focused on the effect of TAS-301 on the PDGF-induced influx of Ca\(^{2+}\), which is one pathway for the increase [Ca\(^{2+}\)], because it was reported that a sustained elevation of Ca\(^{2+}\) influx from the extracellular medium is a prerequisite for PDGF-induced VSMC proliferation (14, 15). From the results using \(^{45}\)Ca, we demonstrated that TAS-301 dose-dependently inhibited the initial uptake of \(^{45}\)Ca after PDGF stimulation. NiCl\(_2\), a blocker of Ca\(^{2+}\) entry (14), also attenuated PDGF-induced \(^{45}\)Ca uptake; on the other hand, nifedipine, a well-known L-type Ca\(^{2+}\) channel blocker, was ineffective, even at 1 μM. These findings agree with previous observations (14). Because it was reported that the expression of L-type voltage-dependent Ca\(^{2+}\) channels is markedly reduced in proliferating VSMC (26), it seems likely that PDGF-induced Ca\(^{2+}\) influx would be mediated by some non-voltage-dependent mechanisms. In agreement with this possibility, it was recently reported that the anti-proliferative action of L-type Ca\(^{2+}\) channel blockers against VSMCs may be due to various effects other than the blockade of voltage-dependent Ca\(^{2+}\) channels such as the inhibition of the expression of immediate-early gene (27). Although the mechanism by which PDGF causes the Ca\(^{2+}\) influx remains to be fully clarified despite extensive studies for many years, Ca\(^{2+}\) influx after receptor stimulation is in general referred to as receptor-operated Ca\(^{2+}\) influx (28). Therefore, TAS-301 might block this receptor-operated Ca\(^{2+}\) influx, which may be an important pathway of Ca\(^{2+}\) influx after PDGF stimulation.

Activation of a signaling pathway involving ERK and PKC has been also suggested to be important for transducing mitogenic signals and to play a key role in PDGF-induced proliferation (7, 8). Therefore, we examined whether TAS-301 would affect PDGF-stimulated ERK and PKC activation. As the results, TAS-301 failed to inhibit ERK, but was capable of inhibiting the PKC activation induced by PDGF. Activation of ERK after PDGF stimulation is known to involve the sequential activation of Ras, Raf-1, and MEK (29). Thus, TAS-301 does not affect the Ras-ERK-mediated signal pathway.

PKC activation can cause induce formation of the AP-1 complex that is thought to play an essential role in cellular proliferation (9). Because TAS-301 could inhibit the PDGF-induced PKC activation, we assumed that TAS-301 would have an inhibitory effect on the induction of AP-1 after stimulation of cells with a PKC activator, PMA. Consistent with this possibility, we showed that TAS-301 inhibited PMA-mediated AP-1 induction. These findings suggest that the anti-proliferative effects of TAS-301 may be, in part, mediated by inhibiting the induction of AP-1 complexes after PKC activation.

Ca\(^{2+}\) is required for several isoforms of PKC, and the Ca\(^{2+}\)/PKC signaling pathway is thought to be one of the signaling systems important in VSMC proliferation (30). This pathway has been traditionally associated with the intracellular signaling of G-protein-coupled receptors having seven membrane spanning domains such as the angiotensin II receptor. However, this pathway is not only used by the above mentioned receptor type but is also activated by membrane tyrosine kinase receptors such as PDGF- and FGF-receptor (31). Therefore, the inhibitory effect of TAS-301 on the Ca\(^{2+}\) influx may be important for inhibiting the PKC activation and following AP-1 induction.

In conclusion, our present study provides evidence that newly synthesized TAS-301 is a potent inhibitor of growth factor-stimulated proliferation in VSMCs. The actions of TAS-301 on VSMC proliferation may be due to the blockade of receptor-operated Ca\(^{2+}\) influx and the inhibition of downstream signals carried by the Ca\(^{2+}\)/PKC signaling pathway, leading to AP-1 induction. Therefore, TAS-301, with its inhibitory effect on the common mitogenic signals activated by multiple growth factors, may have benefit as an anti-proliferative drug and provide a potential therapeutic approach for preventing vascular disorders such as atherosclerosis and restenosis after PTCA.
REFERENCES

3 Lindner V and Reidy MA: Proliferation of smooth muscle cells after vascular injury is inhibited by an antibody against basic fibroblast growth factor. Proc Natl Acad Sci USA 88, 3739 – 3743 (1991)
11 Huang C-L, Takenawa T and Ives HE: Platelet-derived growth factor-mediated Ca2+ entry is blocked by antibodies to phosphatidylinositol 4,5-bisphosphate but does not involve heparin-sensitive inositol 1,4,5-trisphosphate receptors. J Biol Chem 266, 4045 – 4048 (1991)