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Pro-apoptotic effects of imatinib on PDGF-stimulated pulmonary artery smooth muscle cells from patients with idiopathic pulmonary arterial hypertension ☆

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ABSTRACT

Background: Remodeling of the pulmonary artery by an inappropriate increase of pulmonary artery smooth muscle cells (PASMCs) is problematic in the treatment of idiopathic pulmonary arterial hypertension (IPAH). Effective treatment that achieves reverse remodeling is required. The aim of this study was to assess the pro-apoptotic effects of imatinib, a platelet-derived growth factor (PDGF)-receptor tyrosine kinase inhibitor, on PASMCs obtained from patients with IPAH.

Methods: PASMCs were obtained from 8 patients with IPAH undergoing lung transplantation. Cellular proliferation was assessed by ^{3}H-thymidine incorporation. Pro-apoptotic effects of imatinib were examined using TUNEL and caspase-3,7 assays and using transmission electron microscopy.

Results: Treatment with imatinib (0.1 to 10 μg/mL) significantly inhibited PDGF-BB (10 ng/mL)-induced proliferation of PASMCs from IPAH patients. Imatinib (1 μg/mL) did not induce apoptosis in quiescent IPAH-PASMCs, but it had a pro-apoptotic effect on IPAH-PASMCs stimulated with PDGF-BB. Imatinib did not induce apoptosis in normal control PASMCs with or without PDGF-BB stimulation. PDGF-BB induced phosphorylation of Akt at 15 min, and Akt phosphorylation was inhibited by imatinib in IPAH-PASMCs. Akt-I-1/2 (1 μmol/L), an Akt inhibitor, in the presence of PDGF-BB significantly increased apoptotic cells compared with the control condition. Thus, Akt-I-1/2 could mimic the effects of imatinib on PASMCs.

Conclusion: Imatinib has anti-proliferative and pro-apoptotic effects on IPAH-PASMCs stimulated with PDGF. The inhibitory effect of imatinib on Akt phosphorylation induced by PDGF plays an important role in the pro-apoptotic effect.

1. Introduction

Idiopathic pulmonary arterial hypertension (IPAH) is a progressive disease characterized by progressive elevation of pulmonary vascular resistance and pulmonary artery pressure. Increased pulmonary vascular resistance is induced by pulmonary vasoconstriction, vascular remodeling by intimal and medial hypertrophy, and thrombosis [1,2]. Pulmonary vascular medial hypertrophy is caused by an inappropriate increase in pulmonary artery smooth muscle cells (PASMCs). Treatment with several vasodilators such as calcium channel blockers, prostaglandin I_2 and endothelin receptor antagonists was found to improve survival of patients with IPAH, but 5-year survival remains at 50% [3,4]. Effective treatment that achieves reverse remodeling is needed. This will require anti-proliferative and pro-apoptotic agents for PASMCs.

We have reported that platelet-derived growth factor (PDGF)-BB stimulation causes a higher growth rate of cultured PASMCs from patients with IPAH than that of control cells [5–7]. Recently, the use of a PDGF-receptor inhibitor such as imatinib (STI571) is starting to garner attention as a targeted therapy for pulmonary hypertension (PH) [8–11]. Imatinib is a drug used to treat certain types of cancer such as chronic myelogenous leukemia and gastrointestinal stromal tumors. In laboratory settings, imatinib is used as an experimental agent to suppress PDGF by inhibiting PDGF receptor ß (PDGF-Rß). It is an agent that acts by specifically inhibiting a certain enzyme, tyrosine kinase, that

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Table 1
Clinical data of patients with IPAH.

Patient	Time	Sex	Age	PAP (s/d/m) (mmHg)	mRAP (mmHg)	CI (L/min/m^2)	PVR (dyn/s/cm^5)	BNP (pg/dL)
1	Prior to drug therapy	F	7	150/72/98	4	3.8	1918	136
	Prior to transplantation		13	99/59/72	15	2.3	2779	334
2	Prior to drug therapy	F	28	88/40/59	10	1.9	1416	408
	Prior to transplantation		31	73/30/48	1	2.1	1199	325
3	Prior to drug therapy	F	10	118/67/84	14	2	NA	NA
	Prior to transplantation		13	111/49/67	10	1.7	2438	203
4	Prior to drug therapy	F	NA	NA	NA	NA	NA	NA
	Prior to transplantation		28	113/36/66	7	1.8	3340	50
5	Prior to drug therapy	M	16	163/71/106	2	1.7	2267	14
	Prior to transplantation		20	70/40/50	2	3.3	808	18
6	Prior to drug therapy	F	39	74/23/42	3	2.6	NA	NA
	Prior to transplantation		43	107/47/72	15	2.4	3056	622
7	Prior to drug therapy	F	13	96/50/68	4	2.3	1495	411
	Prior to transplantation		16	83/51/65	8	2.5	784	216
8	Prior to drug therapy	M	NA	NA	NA	NA	NA	NA
	Prior to transplantation		11	130/51/80	9	1.9	2629	420
Mean ± SE	Prior to drug therapy		19 ± 5	mPAP: 76 ± 10	6 ± 2	2.4 ± 0.3	1774 ± 198	242 ± 100
	Prior to transplantation		22 ± 4	mPAP: 65 ± 4	8 ± 2	2.3 ± 0.2	2129 ± 366	273 ± 70

M: male, F: female, PAP: pulmonary artery pressure, s/d/m: systolic/diastolic/mean, mRAP: mean right atrial pressure, CI: cardiac index, PVR: pulmonary vascular resistance, BNP: plasma concentration of brain natriuretic peptide, NA: not available.

is characteristic of a particular cancer cell, rather than non-specifically inhibiting the proliferation of and killing all rapidly dividing cells. Schermuly et al. reported that imatinib reverses pulmonary vascular remodeling and cor pulmonale in rats with monocrotaline-induced PH and in mice with chronic hypoxia-induced PH [8]. Perros et al. reported that PDGF-BB-induced proliferation and migration of PASMCs from patients with IPAH were inhibited by imatinib [10].

Not only inhibition of proliferation but also induction of apoptosis of PASMCs is needed to actively reduce stenosis due to vascular remodeling at small pulmonary arteries of patients with IPAH. These two effects may lead to reverse remodeling of the pulmonary vasculature. Expression of PDGF-B is up-regulated in the medial layer of small pulmonary arteries of rats with monocrotaline-induced PH and imatinib induces apoptosis in the small pulmonary arteries [8]. However, imatinib does not induce apoptosis in cultured IPAH-PASMCs without PDGF treatment [10]. Thus, imatinib may not be able to induce apoptosis in quiescent cells. We hypothesized that imatinib in the presence of PDGF-BB induces apoptosis of PASMCs from patients with IPAH, but that imatinib cannot induce apoptosis in PASMCs without PDGF stimulation. We therefore investigated whether imatinib in the presence and absence of PDGF-BB induces apoptosis of PASMCs from patients with IPAH.

Akt is a member of the serine/threonine-specific kinase family known for facilitating cell survival via the inhibition of apoptotic pathways [12]. Therefore, induction of apoptosis of IPAH-PASMCs may be related to Akt inactivation. We also investigated whether imatinib inhibits Akt activation.

2. Materials and methods

2.1. Isolation, culture and identification of PASMCs

Peripheral segments of the pulmonary artery were obtained at lung transplantation [13] from 8 patients with IPAH as previously described [5,6,14,15] (2 males and 6 females; mean age, 22 ± 4 years; age range 11–43 years) (Table 1). For normal control experiments, samples of pulmonary arteries were also obtained at lung lobectomy from a patient with bronchogenic carcinoma (male, 58 years old) who showed no evidence of PAH and received no systemic chemotherapy or radiation therapy before lung lobectomy as previously described [5,6,14,15]. Samples of the pulmonary arteries were obtained from the most distal area from the carcinoma in the resected lobe. All of the studies were approved by the Ethics Committee of Okayama University Graduate School of Medicine, Density, and Pharmaceutical Sciences, and written informed consent was obtained from all patients before the procedure. The investigation also conforms to the principles outlined in the Declaration of Helsinki.

PASMCs were isolated as described previously [5,6,14–16]. Peripheral segments of pulmonary arteries smaller than 1 mm in outer diameter were disaggregated with collagenase and cut into 2-mm-long sections, and then the adventitia and endothelial cell layers were removed. Vessels were plated on a 6-well plate with Dulbecco's modified Eagle's medium (DMEM; Gibco, Grand Island, NY, USA) supplemented with 10% fetal bovine serum (FBS; Sigma) and 0.1 mg/mL kanamycin (Sigma) and incubated in a humidified 5% CO2 atmosphere at 37 °C. The culture medium was changed every 3 days. After reaching confluence, the cells were subcultured by treatment with trypsin

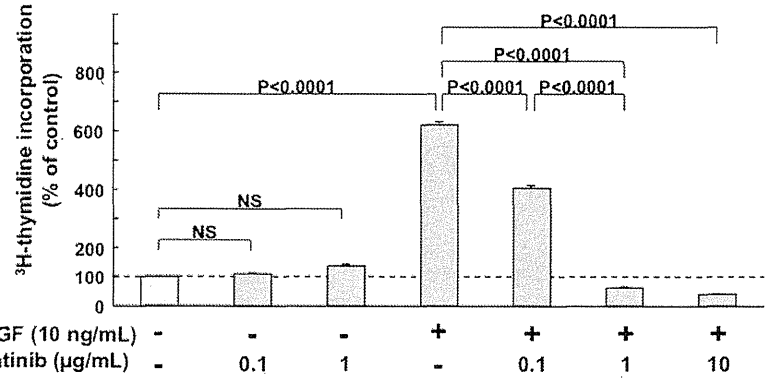

Fig. 1. Inhibitory effect of imatinib on proliferation of PASMCs from IPAH patients. Anti-proliferative effects of imatinib (0.1 to 10 μg/mL) on IPAH-PASMCs stimulated with PDGF-BB (10 ng/mL). ^{3}H-thymidine incorporation was measured. Counts per minute (cpm) were expressed as a percentage of cpm of IPAH-PASMCs treated with a diluent (control). Data are mean ± SE.

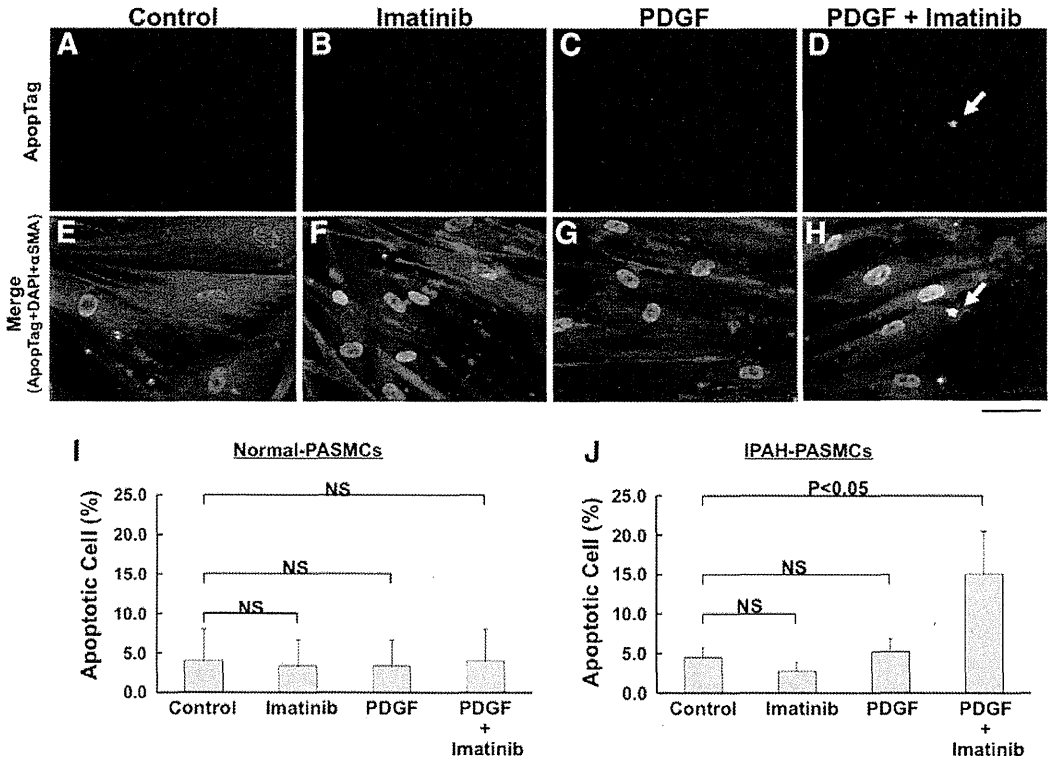

Fig. 2. Effect of imatinib on apoptosis of PASMCs in TUNEL assay by ApopTag fluorescein. A to D, ApopTag fluorescein (green). E to H, Combined images (merge) of ApopTag fluorescein, DAPI (blue) and αSMA (red). A and E, IPAH-PASMCs without treatment. B and F, IPAH-PASMCs treated with imatinib (1 μg/mL). C and G, IPAH-PASMCs treated with PDGF-BB (10 ng/mL). D and H, IPAH-PASMCs treated with imatinib and PDGF-BB. Arrow shows a TUNEL-positive cell (green). Bar = 500 μm. I, Effect of imatinib on apoptosis of normal PASMCs in TUNEL assay. J, Effect of imatinib on apoptosis of IPAH-PASMCs in TUNEL assay. Imatinib (1 μg/mL) in the presence of PDGF-BB (10 ng/mL) significantly increased TUNEL-positive (apoptotic) cells in IPAH-PASMCs compared with the control condition ($P<0.05$). Data are mean ± SE.

(0.05%)/ethylenediaminetetraacetic acid (EDTA) (0.02%). Cell identification was confirmed by the examination of cytoskeletal components (α-smooth muscle actin, myosin, and smoothelin) using an immunocytochemical technique as described previously [5,15]. Cells between passages 3 to 5 were used for all experiments.

2.2. Effects of imatinib on cell proliferation

To assess the antiproliferative effect of imatinib on PASMCs, we measured ^{3}H-thymidine incorporation using methods described previously [5,16]. PASMCs were reseeded in 24-well plates at a density of 5×10^4 cells/well on day 0. After 16 h of incubation (on day 1), the culture media were replaced with low-serum culture media (DMEM, 0.1% FBS, and 0.1 mg/mL kanamycin), and the cultured cells were made quiescent for 48 h. On day 3, PDGF-BB (10 ng/mL) (Sigma), imatinib (0.1 to 10 μg/mL) (Novartis) or an Akt inhibitor, Akt-I-1/2 (1 μmol/L) (Calbiochem), was added to the media. After 21 h (on day 4), the cells were labeled with ^{3}H-thymidine at 1 μCi/mL for 3 h. After completion of labeling, the cells were washed with ice-cold PBS, fixed with 5% trichloroacetic acid and 95% ethanol, and lysed with 200 μL/well of 0.33 mol/L NaOH. Aliquots of the cell lysates were neutralized with 1 mol/L HCl, and the radioactivity was measured in a liquid scintillation analyzer (TRI-CARB 2200CA; Packard, Downers Grove, IL, USA).

2.3. Western blot analysis

PASMCs from patients with IPAH were prepared in the same manner as that described for analysis of DNA synthesis. They were treated in the presence or absence of PDGF-BB (10 or 100 ng/mL), imatinib (1 or 10 μg/mL) and a mitogen-activated protein kinase/extracellular signal-regulated kinase (MEK) inhibitor, U0126 (3 μmol/L) (Promega). Western blot analysis was performed as described previously [5,7]. Briefly, total cell lysates of cultured PASMCs were extracted in commonly used radioimmunoprecipitation (RIPA) buffer with 10 mg/mL phenylmethylsulfonyl fluoride (Sigma) and then concentrated by centrifugation at 12,000 rpm for 20 min. Protein samples (10 μg) were loaded on 10% sodium dodecyl sulfate-polyacrylamide gel and blotted onto nitrocellulose membranes. Blots were incubated with rabbit anti-p27 antibody (Santa Cruz Biotechnology), anti-GAPDH antibody (Chemicon), anti-phospho-Akt antibody and anti-total-Akt antibody (Cell Signaling Technology Inc., Beverly, MA). The relative integrated density of each protein band was digitized by NIH image J 1.34 s.

2.4. Evaluation of apoptosis

TUNEL assays were performed using an ApopTag fluorescein in situ apoptosis detection kit (Chemicon International Inc.) according to the manufacturer's instructions as described previously [17]. Nuclear morphology was examined by labeling with DAPI solution (0.6 μg/mL, Dojindo Laboratories). Immunofluorescence staining was performed to confirm α-smooth muscle actin (αSMA) expression using αSMA antibody (1:100 dilution, Sigma). Caspase assay was performed using a CaspaTag Caspase-3/7 in situ apoptosis detection kit (Chemicon International Inc.) according to the manufacturer's instructions. Nuclear morphology was examined by Hoechst staining. The samples were analyzed by fluorescence microscopy (Olympus IX71, Olympus Optical Co. Ltd, Tokyo, Japan). For each cover slip, 5–10 fields (with 10–30 cells in each field) were randomly selected to determine the percentage of apoptotic cells in total cells based on the morphological characteristics of apoptosis. PASMCs were reseeded on collagen-coated glass cover slips in 12-well plates at a density of 5×10^4 cells/well on day 0. After 16 h of incubation (on day 1), the culture media were replaced with low-serum culture media (DMEM, 0.1% FBS, and 0.1 mg/mL kanamycin), and the cultured cells were made quiescent for 48 h. On day 3, PDGF-BB (10 ng/mL), imatinib (1 μg/mL) or Akt-I-1/2 (1 μmol/L) was added to the media. After 24 h (on day 4), the cells were stained by using an ApopTag fluorescein in situ apoptosis detection kit or CaspaTag in situ apoptosis detection kit.

Transmission electron microscopy was performed with an electron microscope (H-7100: Hitachi; Tokyo, Japan).

To observe cellular apoptosis with a time-lapse system (Olympus Optical Co.), PASMCs were cultured on a 35-mm culture dish that has a micro-photolithographed squared pattern (Kuraray Co., Ltd., Tsukuba, Japan) [7] so that the apoptotic cells will not disappear from view.

2.5. Statistical analysis

All results are expressed as mean ± SE. Statistical significance for comparison between the two measurements was determined using Student's *t* test. For comparison between the different treatment groups, statistical analysis was performed using one-

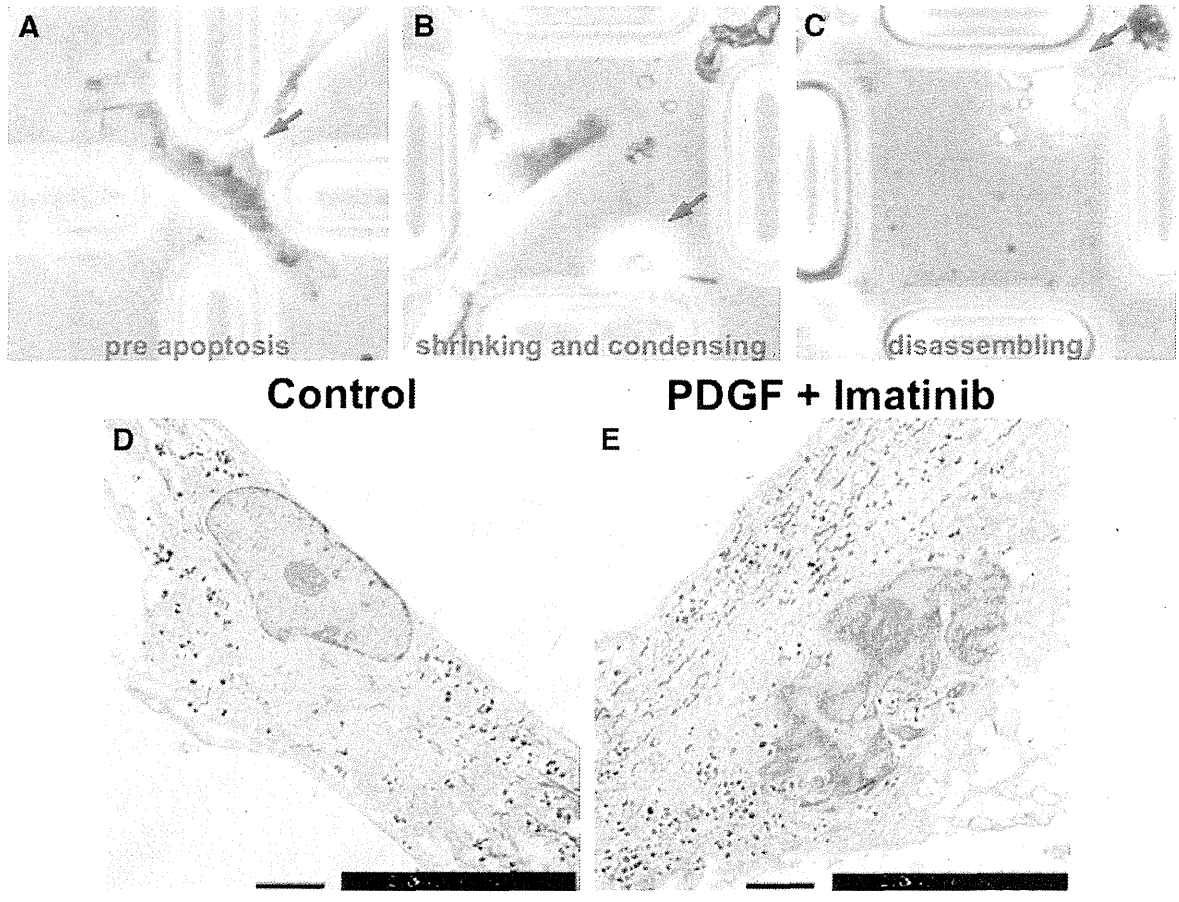

Fig. 3. Effect of imatinib on apoptosis of PASMCs in time-lapse microscopy and transmission electron microscopy. A to C, Representative images of time-lapse microscopy. IPAH-PASMCs were treated with imatinib (1 μg/mL) and PDGF-BB (10 ng/mL). Bar = 20 μm. D and E, Representative images of transmission electron microscopy. D, IPAH-PASMCs without treatment (control). E, IPAH-PASMCs treated with imatinib (1 μg/mL) and PDGF-BB (10 ng/mL). Bar = 5 μm.

way ANOVA with Fisher's PLSD test. Values of $P<0.05$ were considered to be statistically significant.

3. Results

3.1. Inhibitory effect of imatinib on proliferation of PASMCs from IPAH patients

Treatment with imatinib inhibited PDGF-BB-induced proliferation of PASMCs from IPAH patients as assessed by ^{3}H-thymidine incorporation (n = 5–12 experiments in each cell) (Fig. 1). This result is consistent with recent findings of other investigators [10].

3.2. Effect of imatinib on apoptosis of PASMCs from IPAH patients

We performed a TUNEL assay using an ApopTag fluorescein to assess the effect of imatinib on apoptosis of PASMCs from IPAH patients. Fig. 2 shows representative cases of the TUNEL assay. TUNEL-positive cell (green) was observed after 24-hour treatment with imatinib (1 μg/mL) in the presence of PDGF-BB (10 ng/mL) (Fig. 2D and H). However, imatinib (1 μg/mL) (Fig. 2B and F) or PDGF-BB (10 ng/mL) (Fig. 2C and G) alone did not induce apoptosis in IPAH-PASMCs. Imatinib (1 μg/mL) in the presence of PDGF-BB (10 ng/mL) significantly increased TUNEL-positive cells in IPAH-PASMCs compared with the control condition in IPAH-PASMCs ($P<0.05$: 15.1 ± 5.4% versus 4.5 ± 1.3%, n = 4 or 5 experiments in each cell line) (Fig. 2J). There was no significant difference in the percentage of TUNEL-positive cells between the imatinib alone or PDGF alone condition and the control condition (Fig. 2J). There was also no significant difference between the imatinib alone, PDGF alone or both imatinib and PDGF condition and the control condition in normal PASMCs (P = NS, n = 5 experiments) (Fig. 2I).

Fig. 3A, B and C shows the apoptosis induced by the combination of imatinib (1 μg/mL) and PDGF-BB (10 ng/mL) in IPAH-PASMCs as assessed by time-lapse microscopy. A PASMC shows shrinking and condensing and finally disassembling. Fig. 3E shows a transmission electron microscopic image of an apoptotic cell in IPAH-PASMCs. Condensation of chromatin along the nuclear membrane and fragmentation of the nucleus were observed in cultured IPAH-PASMCs treated with imatinib (1 μg/mL) and PDGF-BB (10 ng/mL).

Fig. 4 shows representative cases of the caspase assay in IPAH-PASMCs. Caspase-3 and −7-active cell was observed after 24-hour treatment with imatinib (1 μg/mL) in the presence of PDGF-BB (10 ng/mL) (Fig. 4D and H). Imatinib (1 μg/mL) in the presence of PDGF-BB (10 ng/mL) significantly increased caspase-3 and −7-active cells in IPAH-PASMCs compared with the control condition ($P<0.01$: 12.4 ± 3.0% versus 2.2 ± 1.2%, n = 5 experiments in each cell line) (Fig. 4J). There was no significant difference in the percentage of caspase-3 and −7-positive cells between the imatinib alone or PDGF alone condition and the control condition in IPAH-PASMCs (Fig. 4J). There was also no significant difference between the imatinib alone, PDGF alone or both imatinib and PDGF condition and the control condition in normal PASMCs (P = NS, n = 5 experiments) (Fig. 3I).

These results show that imatinib did not induce apoptosis in normal PASMCs and quiescent IPAH-PASMCs but that imatinib had a proapoptotic effect on IPAH-PASMCs stimulated with PDGF.

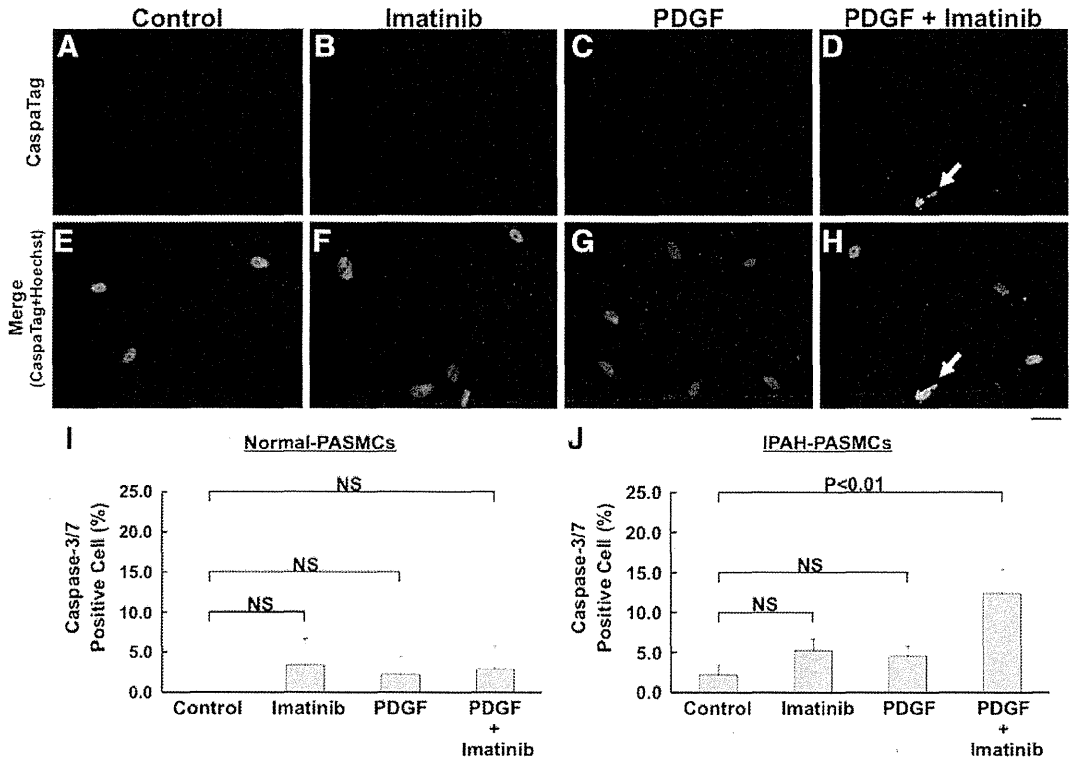

Fig. 4. Effect of imatinib on apoptosis of PASMCs in Caspase assay using a CaspaTag Caspase-3/7 in situ apoptosis detection kit. A to D, CaspaTag staining (green). E to H, Combined images (merge) of CaspaTag staining and Hoechst nuclear staining (blue). A and E, IPAH-PASMCs without treatment. B and F, IPAH-PASMCs treated with imatinib (1 μg/mL). C and G, IPAH-PASMCs treated with PDGF-BB (10 ng/mL). D and H, IPAH-PASMCs treated with imatinib and PDGF-BB. Arrow shows a caspase-3/7-positive cell (green). Bar = 500 μm. I, Effect of imatinib on apoptosis of normal PASMCs in Caspase assay. J, Effect of imatinib on apoptosis of IPAH-PASMCs in Caspase assay. Imatinib (1 μg/mL) in the presence of PDGF-BB (10 ng/mL) significantly increased caspase-positive (apoptotic) cells in IPAH-PASMCs compared with the control condition ($P<0.01$). Data are mean ± SE.

3.3. Effect of imatinib on PDGF-BB-induced phosphorylation of Akt

Western blot analysis revealed that PDGF-BB induced phosphorylation of Akt at 15 min (Fig. 5A, lanes 2 and B). Akt phosphorylation was significantly inhibited by imatinib (1 ng/mL) compared with the treatment with PDGF-BB ($P<0.05$, $n=4$ experiments) (Fig. 5A, lanes 3 and B).

Akt-I-1/2 (1 μmol/L), an Akt inhibitor, could mimic the effects of imatinib on PASMCs. Akt-I-1/2 significantly inhibited PDGF-induced proliferation of IPAH-PASMCs as assessed by ^{3}H-thymidine incorporation ($P<0.001$, $n=10$ experiments) (Fig. 5C). Akt-I-1/2 in the presence of PDGF-BB significantly increased TUNEL-positive cells ($P<0.05$, $n=5$ experiments) (Fig. 5D) and caspase-3,7-positive cells in IPAH-PASMCs ($P<0.05$, $n=5$ experiments) (Fig. 5E) compared with the control condition. These results show that the inhibition of Akt is strongly related to the anti-proliferative and pro-apoptotic effects of imatinib on PDGF-stimulated IPAH-PASMCs.

4. Discussion

Two major new findings were obtained in the present study. First, imatinib did not induce apoptosis in quiescent IPAH-PASMCs and normal PASMCs, but it had a pro-apoptotic effect on IPAH-PASMCs stimulated with PDGF. Second, inhibition of Akt is related to the anti-proliferative and pro-apoptotic effects of imatinib on PDGF-stimulated IPAH-PASMCs.

Imatinib alone did not induce apoptosis in IPAH-PASMCs. This result is consistent with recent findings of other investigators [10]. However, the combination of imatinib and PDGF induced apoptosis. Therefore, imatinib did not induce apoptosis in quiescent IPAH-PASMCs, but it had a pro-apoptotic effect on IPAH-PASMCs stimulated with PDGF. It has been reported that PDGF-A and PDGF-B mRNA levels were increased in small pulmonary arteries from patients with IPAH [10] and that serum PDGF-BB levels across the lung circulation were higher in IPAH patients [18]. Therefore, imatinib is expected to induce apoptosis in clinical settings. Further studies are needed to clarify this point.

Many signaling pathways, including ERK, p38 MAPK and Akt, are involved in proliferation and survival of PASMCs [14,19]. Akt is a member of the serine/threonine-specific kinase family known for facilitating cell survival via the inhibition of apoptotic pathways. It has been shown that PDGF stimulation transiently phosphorylates Akt and the mammalian target of rapamycin (mTOR) in PASMCs from patients with chronic thromboembolic pulmonary hypertension [19]. In our study, PDGF-BB induced phosphorylation of Akt and it was inhibited by imatinib in IPAH-PASMCs. Akt-I-1/2, an Akt inhibitor, could mimic the effects of imatinib on PASMCs. Akt is related to the anti-proliferative and pro-apoptotic effects of imatinib on PDGF-stimulated IPAH-PASMCs.

Imatinib is a drug used for treating chronic myelogenous leukemia and gastrointestinal stromal tumors. However, resistance to imatinib can occur [20–22]. Not only primary resistance within the first two months but also secondary resistance develops after a median of about 2 years of treatment with the drug. Hatano et al. reported that imatinib decreases the plasma concentration of PDGF-BB in patients with PAH, while the improvement in hemodynamic parameters is transient [11]. We showed that imatinib had a pro-apoptotic effect on IPAH-PASMCs stimulated with PDGF in the present study. Thus, imatinib would induce apoptosis only in the early period of treatment when plasma PDGF-BB levels are relatively high. After the PDGF levels have decreased, imatinib would not be able to induce apoptosis. Therefore, resistance to imatinib might occur in patients with pulmonary hypertension. Attention is needed in clinical use.

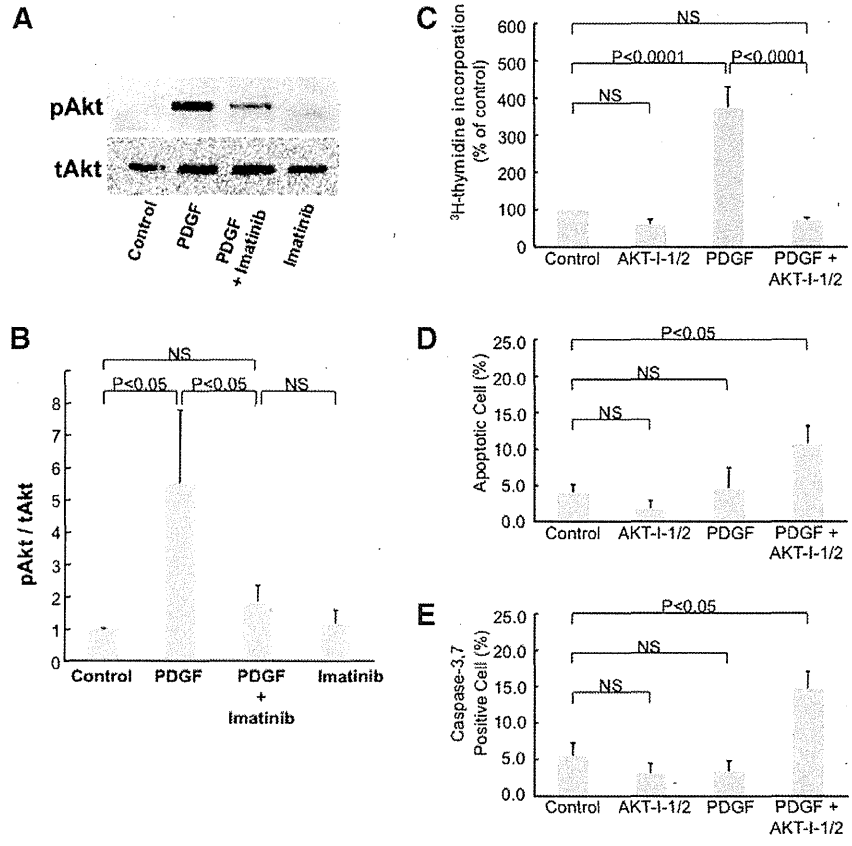

Fig. 5. Effect of imatinib on PDGF-BB-induced phosphorylation of Akt and effects of an Akt inhibitor on PDGF-BB-stimulated proliferation and apoptosis of PASMCs. A, Western blot analysis of total Akt (tAkt) and phosphorylated Akt (pAkt). PDGF-BB (10 ng/mL) induced phosphorylation of Akt at 15 min (lanes 2). Akt phosphorylation was inhibited by imatinib (1 ng/mL) (lanes 3). B, Bar graphs show semiquantitive analysis of pAkt expression level in IPAH-PASMCs. Data are mean ± SE of the intensity of the band corresponding to pAkt relative to tAkt. C, Anti-proliferative effect of Akt-I-1/2 (1 μmol/L), an Akt inhibitor, on IPAH-PASMCs stimulated with PDGF-BB (10 ng/mL). ^{3}H-thymidine incorporation was measured. Counts per minute (cpm) were expressed as a percentage of cpm of IPAH-PASMCs treated with a diluent (control). Data are mean ± SE. D, Effect of Akt-I-1/2 (1 μmol/L) on apoptosis of PASMCs in TUNEL assay by ApopTag fluorescein. E, Effect of Akt-I-1/2 (1 μmol/L) on apoptosis of PASMCs in Caspase assay using a CaspaTag Caspase-3/7 in situ apoptosis detection kit.

In conclusion, imatinib inhibited PDGF-induced proliferation of IPAH-PASMCs. Imatinib did not induce apoptosis in quiescent IPAH-PASMCs, but it had a pro-apoptotic effect on IPAH-PASMCs stimulated with PDGF. Inhibition of Akt may be important in the anti-proliferative and pro-apoptotic effects of imatinib on PDGF-stimulated IPAH-PASMCs. Modulation of PDGF signaling such as Akt is important. Inhibition of PDGF signaling by imatinib may become a useful molecular-targeted therapy for IPAH.

Conflict of interest

There are no relationships with industry.

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