Vascular Medicine

Effects of HIV Protease Inhibitors on Progression of Monocrotaline- and Hypoxia-Induced Pulmonary Hypertension in Rats

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Background—Pulmonary hypertension (PH) is among the complications of HIV infection. Combination antiretroviral therapy may influence the progression of HIV-related PH. Because Akt signaling is a potential molecular target of HIV protease inhibitors (HPIs), we hypothesized that these drugs altered monocrotaline- and hypoxia-induced PH in rats by downregulating the Akt pathway, thereby inhibiting pulmonary artery smooth muscle cell proliferation.

Methods and Results—Daily treatment with each of 3 first-generation HPIs (ritonavir 30 mg/kg, amprenavir 100 mg/kg, and nelfinavir 500 mg/kg) started 3 weeks after a subcutaneous monocrotaline injection (60 mg/kg) substantially diminished pulmonary artery pressure, right ventricular hypertrophy, number of muscularized pulmonary vessels, pulmonary arterial wall thickness, and proliferating pulmonary vascular Ki67-labeled cells without affecting vessel caspase 3 staining. HPI treatment partially prevented the development of hypoxia- and monocrotaline-induced PH. Monocrotaline-induced PH was associated with marked activation of Akt signaling in the lungs and proximal pulmonary arteries, with increases in phosphorylated Akt, phosphorylated glycogen-synthase-kinase-3β (GSK3), and phosphorylated endothelial nitric oxide synthase, all of which decreased markedly after treatment with each HPI. In contrast, PH-associated increases in phosphorylated extracellular signal-related kinase 1/2 and myosin light-chain phosphatase were unaltered by the HPIs. The 3 HPIs and the phosphatidylinositol 3-kinase inhibitor LY294002 inhibited platelet-derived growth factor–induced phosphorylation of Akt and GSK3 in cultured pulmonary artery smooth muscle cells and blocked cell proliferation; this last effect was abolished by the GSK3 inhibitor SB216763.

Conclusion—These results support an effect of HPIs on pulmonary vascular remodeling mediated by inhibition of Akt phosphorylation and consequently of pulmonary artery smooth muscle cell proliferation. (Circulation. 2010;122:1937-1947.)

Key Words: AIDS ■ muscle, smooth ■ pulmonary heart disease ■ remodeling

Pulmonary hypertension (PH) can occur as a life-threatening vascular complication of HIV infection.1 Survival gains in HIV-infected patients have translated into an increase in the prevalence of PH, which has been estimated at 0.5% in this population, ie, ~1000-fold higher than the prevalence of idiopathic PH in the general population.2,3 HIV infection may account for ~7% of all PH cases and 10% of cases accompanied by portal hypertension.2,3 The clinical presentation and underlying pathology of HIV-related PH are similar to those of idiopathic or associated PH, with marked structural remodeling of the pulmonary vessels leading to an increase in pulmonary vascular resistance.4

Clinical Perspective on p 1947

The prevalence of HIV-related PH seems unchanged compared with the 1990s, before the introduction of highly active antiretroviral therapy.2,3 However, the potential impact of combination antiretroviral therapy on the progression of HIV-related PH is still under investigation, and the effects of antiretroviral drugs on pulmonary function and hemodynamics remain controversial. Highly active antiretroviral therapy was associated with improved hemodynamics and survival in some studies5,6 and with improved exercise tolerance but unchanged pulmonary hemodynamics in oth-
ers.7,8 The effect of highly active antiretroviral therapy in these studies showed considerable interindividual variability, with some patients achieving near-normal pulmonary artery pressures and others experiencing either no change or a further pressure increase.7–9 Thus, an important and still unresolved question is whether antiretroviral therapy, more specifically HIV protease inhibitors (HPIs), interfere with pathophysiological processes underlying the pulmonary vascular remodeling that occurs during PH progression.

HPIs are peptidomimetics that inhibit the HIV enzyme aspartyl protease required to produce infectious viral particles.10 Interference of HPIs with host-cell functions leads to a number of side effects such as insulin resistance, diabetes mellitus, and dyslipidemia.11 HPIs have been shown to inhibit cancer cell growth in vitro and cancer growth in vivo in experimental animals.12–14 One possible mechanism of this growth-inhibiting effect and perhaps also of the insulin resistance-inducing effect is inhibition of the Akt signaling pathway.12–14 In pulmonary artery smooth muscle cells (PA-SMCs), Akt signaling is a downstream target of several factors such as platelet-derived growth factor (PDGF)15,16 and serotonin,17 which are important mediators of the pulmonary vascular remodeling process.18,19 Because PH is primarily a proliferative disease and because the Akt pathway has been shown in vitro to mediate SMC proliferation via phosphorylation and inactivation of its downstream effector glycogen-synthase-kinase-3β (GSK3β),20 we hypothesized that HPIs slowed PH progression by interfering with the Akt signaling pathway.

We therefore designed the present study to investigate whether 3 first-generation HPIs (ritonavir, amprenavir, and nelfinavir) altered monocrotaline- and hypoxia-induced PH in rats; whether alterations in the Akt signaling pathway occurred during PH progression; and whether the effects of HPIs on the Akt signaling pathway decreased PA-SMC growth, thereby diminishing pulmonary vascular remodeling. In parallel, we investigated the potential effects of HPIs on the mitogen-activated protein kinase and RhoA signaling pathways by measuring the phosphorylation of extracellular signal-related kinase 1/2 and myosin light-chain phosphatase (MYPT).

Methods

Animal Model and Experimental Design

All experiments were performed in adult male Wistar rats (200 to 250 g) according to institutional guidelines complying with national and international regulations. PH was induced by administering 60 mg/kg monocrotaline (Sigma-Aldrich, Lyon, France) subcutaneously or by exposing rats to 10% O2 in a ventilated chamber (Biospherix, New York, NY) for 21 days. To assess the potential curative effects of HPIs, rats given monocrotaline were left untreated for 21 days and then randomly assigned to treatment with ritonavir (30 mg/kg), amprenavir (100 mg/kg), nelfinavir (500 mg/kg), or vehicle from day 21 to 42 (10 animals in each group). All treatments were given by gavage once a day except for nelfinavir, which was given twice daily. The ritonavir dose was close to that used in humans; the amprenavir dose was selected on the basis of information from the manufacturer to obtain in rats the plasma levels achieved in humans; and the nelfinavir dose was selected on the basis of preliminary studies showing decreased effectiveness with lower doses. Rats in each group were euthanized on days 28 and 42 for assessments of treatment effects after 1 and 3 weeks. In another series of experiments, rats were treated with HPIs for 3 weeks either after monocrotaline injection or during hypoxia exposure and then euthanized for assessment of PH. Additional studies were performed at various times after monocrotaline injection for assessments of Akt signaling during PH development.

Assessment of PH

Rats were anesthetized with ketamine (60 mg/kg IM) and xylazine (3 mg/kg IM). A polyvinyl catheter was introduced into the right jugular vein and pushed through the right ventricle into the pulmonary artery.21 After measurement of pulmonary arterial pressure (PAP) and systemic arterial pressure, the thorax was opened and the left lung and proximal pulmonary arteries were immediately removed and frozen in liquid nitrogen for protein immunoblotting. The heart was dissected and weighed for calculation of the right ventricular hypertrophy index (ratio of right ventricular free wall weight over the sum of the septum plus left ventricular free wall weight: RV/LV+S). The right lung was fixed in the distended state with formalin buffer. After paraffin embedding, 5-µm-thick lung sections were stained with hematoxylin-phloxin-saffron. In each rat, the distribution and degree of artery muscularization were assessed by categorizing 40 to 60 intra-acinar arteries as muscular, partially muscular, or nonmuscular. Normalized arterial wall thickness was calculated as the ratio of the difference between the external and internal diameters to the external diameter of the pulmonary artery.

Evaluation of In Situ PA-SMC Death and Proliferation

Proliferation and apoptosis of PA-SMCs were assessed with Ki67 and caspase 3 immunostaining. Tissue sections were deparaffinized in xylene, treated with a graded series of alcohol washes, rehydrated, and incubated in citrate buffer (0.01 mol/L, pH 6) at 90°C for 20 minutes. Endogenous peroxidase activity was blocked with 3% H2O2 and 10% methanol in PBS for 10 minutes. Slides were first incubated for 60 minutes in 1% BSA and 5% goat serum in PBS and then incubated overnight with anti-Ki67 rabbit antibody (1:500, Abcam, Cambridge, Mass) and anti--caspase 3 active rabbit antibody (1:200, R&D Systems, Minneapolis, Minn). The slides were incubated with biotinylated goat anti-rabbit antibody (1:200, Vector Labs, Burlingame, Calif), and the signal was then developed with an immunoperoxidase reagent (ABC-HRP, Vector Labs) and DAB (Sigma-Aldrich) as the substrate.

Effects of HPIs on Isolated PA-SMC Proliferation and Apoptosis

PA-SMCs from rat pulmonary arteries were cultured and characterized as previously described.22 Before treatment, PA-SMCs were placed for 24 hours in Dulbecco modified Eagle medium with 0.1% serum. The cells were exposed to ritonavir, amprenavir, nelfinavir, the selective phosphatidylinositol 3-kinase (PI3K) inhibitor LY294002, or in serum-free medium for 24 hours and then treated with PDGF-BB (10 ng/mL). After 48 hours, tetrazolium salt (MTT; Sigma) was added to each well (0.2 mg/mL). After 4 hours of incubation at 37°C, the culture medium was removed and formazan crystals were solubilized by adding 100 µL dimethyl sulfoxide. Tetrazolium salt reduction to formazan within the cells was quantified by spectrophotometry at 520 nm and taken as an indicator of the number of cells.

Annexin V flow cytometry was performed with a commercially available annexin V–FITC assay (Sigma-Aldrich). Cells were trypsinized and resuspended in media at 1×106 cells per 1 mL and then incubated with annexin V–FITC–conjugated antibody and stained with propidium iodide according to the manufacturer’s instructions. Annexin V staining and propidium iodide staining were detected by fluorescence-activated cell sorting (Becton Dickinson, Franklin Lakes, NJ). Apoptotic cells were propidium iodide–positive cells and annexin V/propidium iodide–positive cells.

Western Blot Analysis

Akt, GSK3, Erk 1/2, MYPT, and endothelial nitric oxide synthase (eNOS) proteins were detected and measured in lung tissues and/or
cells with Western blotting. Total protein from lung tissue (60 g) or cell sample (30 g) was subjected to 10% SDS-PAGE, and the separated proteins were transferred onto polyvinylidene difluoride membranes (Millipore, Molsheim, France). After incubation in blocking solution (TBS/5% milk), the membranes were incubated sequentially with the following antibodies: anti–phosphorylated-(p-)Akt, anti-Akt, anti–p-GSK3, anti-GSK3, anti-Erk 1/2, and anti–p-Erk 1/2 (Cell Signaling Technology, Boston, Mass); anti-eNOS and anti–p-eNOS1177 (BD Transduction Laboratories, Franklin Lakes, NJ); anti-MYPT1 and anti–p-MYPT1Thr696 (Upstate, Molsheim, France); and anti–α-actin (Sigma, Saint-Quentin-Fallavier, France). Densitometric quantification was normalized for the α-actin level in each sample (Gene Tools, Ozyme, Montigny le Bretonneux, France).

Drugs

Amprenavir, a gift from GlaxoSmithKline (Les Ulis, France), was supplied as a powder to be dissolved in dimethyl sulfoxide. Ritonavir and nelfinavir were obtained from the pharmacy of the Henri Mondor Teaching Hospital, Créteil, France. Ritonavir came as gelatin capsules, which were punctured to enable recovery of the content, a viscous liquid that was dissolved in 100% ethanol to produce a concentrated stock solution for use in the experiments. Nelfinavir was available as solid caplets that were ground into a fine powder and then dissolved in water or PBS. The PI3K inhibitor LY924002 was obtained from Calbiochem (San Diego, Calif), and the GSK3 inhibitor SB216763 was purchased from Sigma.

Statistical Analyses

The data are described as mean±SEM. Parametric tests were used after verification that the variables in each group were normally distributed. Two-way ANOVA was performed to compare HPI treatment effects at various times after monocrotaline administration. Comparisons of treatments (3 HPIs and vehicle) at a given time point were performed with 1-way ANOVA. One-way ANOVA was also used to evaluate the time-dependent effects of monocrotaline administration on Akt signaling and to compare data from isolated PA-SMCs treated with various HPI doses. When a significant difference was found, group means were compared by use of the modified t test. Values of P<0.05 were considered significant.
Results

Effects of HPIs on the Progression of Monocrotaline-Induced PH

Rats examined 21 days after monocrotaline administration had severe PH with marked increases in PAP, RV/LV+S, and pulmonary artery muscularization compared with vehicle-treated rats (Figures 1 and 2). RV/LV+S, and pulmonary artery muscularization compared with vehicle-treated rats (Figures 1 and 2). Daily administration of ritonavir (30 mg/kg), amprenavir (100 mg/kg), or nelfinavir (500 mg/kg) from day 21 to 42 after monocrotaline injection led to marked decreases in PAP, right ventricular hypertrophy, number of muscularized pulmonary vessels, and pulmonary arterial wall thickness compared with vehicle-treated rats (Figures 1 and 2). Treatment with any of the 3 HPIs also markedly reduced the number of Ki67-stained cells in the media of remodeled pulmonary vessels without affecting caspase 3 staining (Figure 3). The decrease in Ki67-stained cells was already apparent on day 28, after only 1 week of HPI treatment, compared with vehicle-treated animals. Rats treated with the 3 HPIs did not differ from vehicle-treated rats with respect to systemic arterial pressure and heart rate (data not shown).

Studies were also performed to assess the preventive effects of HPIs on PH development. Treatment of rats with HPIs during exposure to chronic hypoxia or after monocrotaline injection attenuated the development of PH as judged on PAP, right ventricular hypertrophy, and pulmonary artery muscularization (Figure 4).

Inhibition of Akt Signaling by HPIs in Lungs and Proximal Pulmonary Arteries From Monocrotaline-Treated Rats

Although large amounts of Akt and GSK3 protein were found in the lungs and proximal pulmonary arteries of the control animals, only traces of p-Akt and p-GSK3 were detected. Monocrotaline administration was followed by a gradual p-Akt increase in the lungs and proximal pulmonary arteries, with no change in total Akt (Figure 5A). Similarly, p-GSK3 levels increased gradually in the proximal pulmonary arteries, reaching a peak on day 42 (Figure 5A). Lung p-Akt levels were also increased in chronically hypoxic rats compared with normoxic rats (data not shown). Treatment with ritonavir, amprenavir, or nelfinavir from day 21 to 42 after monocrotaline injection was associated with a large decrease in lung p-Akt and with considerably lower p-Akt and p-GSK3 levels in the proximal pulmonary arteries compared with the monocrotaline-injected vehicle-treated controls (Figure 5B). Compared with the monocrotaline-injected rats treated with vehicle instead of an HPI, the rats given 1 week of HPI treatment (day 28 after the monocrotaline injection) had lower p-Akt levels in the lungs and proximal pulmonary arteries despite only small changes in pulmonary vessel muscularization. Similarly, lung p-eNOS levels, which increased in monocrotaline-treated rats compared with control rats, were markedly reduced by treatment with the 3 HPIs (Figure 6A). In contrast, p-Erk 1/2 and p-MYPT levels, which increased in the pulmonary arteries and lungs, respectively, from monocrotaline-treated rats, showed no significant changes in response to HPI treatment (Figure 6B and 6C).

Inhibition of Akt Signaling by HPIs in Cultured PA-SMCs

Pretreatment of PA-SMCs with ritonavir, amprenavir, or nelfinavir for 24 hours led to dose-dependent inhibition of PDGF-induced cell proliferation. Inhibition was complete with 20 μmol/L ritonavir, 10 μmol/L amprenavir,
50 μmol/L nelfinavir, or 30 μmol/L LY924002 (Figure 7A). Increasing the ritonavir concentration resulted in cell toxicity, as shown by the decrease in MTT-labeled cells below the pre-PDGF count with a simultaneous increase in the apoptotic cell count. At doses below the toxic level, ritonavir, amprenavir, and LY924002 inhibited growth without increasing apoptosis, whereas nelfinavir at the highest concentration of 50 μmol/L increased the apoptotic cell count (Figure 7A). Cell pretreatment with amprenavir, nelfinavir, ritonavir, or LY924002 dose-dependently inhibited PDGF-induced phosphorylation of Akt and GSK3 (Figure 7B). Phosphorylation of Akt or GSK3 was completely inhibited by ritonavir, nelfinavir, or LY924002; in contrast, even the highest amprenavir dose only partially inhibited the phosphorylation of Akt (by 20%) and GSK3 (by 50%). These HPI-induced changes in p-Akt and p-GSK3 were not accompanied by changes in nonphosphorylated Akt or GSK3 (Figure 7B). Because p-GSK3 is inactive, we investigated whether inhibition of the active (nonphosphorylated) form of GSK3 abolished the growth-inhibiting effects of HPIs or LY924002 on PA-SMCs stimulated with PDGF. Treatment of the cells with the GSK3 inhibitor SB216763 completely abolished the inhibitory effect of amprenavir, nelfinavir, and LY924002 and almost completely abolished the inhibitory effect of ritonavir (Figure 8).

**Discussion**

Our main finding is that the HPIs ritonavir, amprenavir, and nelfinavir reversed or attenuated the progression of PH induced in rats by monocrotaline injection or hypoxia exposure. In both models, PH development was associated with marked activation of the Akt signaling pathway in the lungs and proximal pulmonary arteries. Ritonavir, amprenavir, or nelfinavir given to monocrotaline-injected rats inhibited Akt phosphorylation in the lungs and pulmonary arteries and decreased the number of muscularized pulmonary vessels without altering the p-Erk or p-MYPT levels. In addition, the 3 HPIs inhibited PDGF-induced phosphorylation of Akt and GSK3 in cultured PA-SMCs and blocked PA-SMC proliferation. Taken together, these results support the ability of HPIs to interfere with pulmonary vascular remodeling by inhibiting Akt phosphorylation and consequently PA-SMC proliferation.

That HPIs may interfere with the PI3K-Akt axis was suggested by several previous studies of tumor cells. The PI3K-Akt axis is a major pathway for cell proliferation and cancer. This pathway is often activated in tumor cells but not in normal host cells and may therefore hold considerable promise as a target for future treatments against cancer. PH is primarily a proliferative disorder that shares several features with cancer. We reasoned that the Akt pathway may be activated in PH and that HPIs may interact with the pulmonary vascular cell proliferation that underlies PH progression. We found that the development of monocrotaline- and hypoxia-induced PH in rats was associated with marked activation of the Akt-GSK3 axis, as shown by increased phosphorylation of Akt and GSK3 in the lungs and proximal pulmonary arteries. Akt activation occurred early during PH...
development, within the first week after the monocrotaline injection, concomitantly with induction of the PA-SMC proliferation that underlies the structural remodeling of pulmonary vessels. Cultured rat PA-SMCs in the quiescent state showed no Akt pathway activation but exhibited marked PDGF-induced phosphorylation of Akt and GSK3. Among the various intracellular downstream effectors of Akt, GSK3 phosphorylation and inactivation are considered the main mechanisms controlling cell proliferation. Our data are consistent with this concept in that PA-SMC treatment with LY294002, a specific PI3K inhibitor that blocked PDGF-induced phosphorylation of Akt and GSK3, completely abolished the growth-promoting effect of PDGF. Furthermore, studies of human PA-SMCs showed that PI3K activity was both necessary and sufficient to mediate mitogen-induced cell proliferation and migration. Interestingly, the LY294002 doses used in our study to block Akt and to inhibit PA-SMC growth did not induce apoptosis. Taken together, these findings indicate clearly that activation of the Akt/GSK3 signaling pathway is a major contributor to PA-SMC growth and that rats given monocrotaline or exposed to hypoxia exhibit marked activation of this pathway.

The main goal of this study was to investigate the ability of 3 HPIs to affect pulmonary vascular remodeling in vivo. In rats with established monocrotaline-induced PH, daily HPI treatment started 3 weeks after the monocrotaline injection produced large decreases in PAP, right ventricular hypertrophy, number of muscularized pulmonary vessels, and pulmonary arterial wall thickness compared with monocrotaline-injected vehicle-treated rats. Similar reductions in these

![Figure 4](image-url)
parameters were obtained when the drugs were given preventively, before the onset of PH, to rats exposed to monocrotaline or hypoxia. In monocrotaline-treated rats with established PH, ritonavir, amprenavir, or nelfinavir given for only 1 week significantly decreased pulmonary arterial wall thickness and the number of Ki67-labeled cells in the pulmonary vascular wall without inducing major changes in caspase 3 staining. These in vivo results are consistent with a main effect of HPIs mediated by cell proliferation inhibition rather than by apoptosis induction. The absence of a proapoptotic effect probably explains why none of the HPIs tested induced complete PH reversal. Although the partial nature of the reversal seen with HPI treatment might be ascribable to the use of insufficient dosages, increasing the dosage was limited by poor tolerance, especially with ritonavir. Moreover, the dosages used in our study replicate those given to human patients and may therefore reflect the effects of the serum concentrations achieved clinically. The effect on PH progression was not markedly different across the 3 HPIs used in our study, and inhibition of pulmonary vascular remodeling by HPIs may therefore constitute a class effect. Moreover, our results suggest that the 3 drugs may act on pulmonary vascular remodeling via a common mechanism.

Because regulation of cell proliferation and motility is a critical step in pulmonary vascular remodeling, researchers are focusing on the development of treatments that specifically target PA-SMC proliferation. Drugs targeting the PIK3-Akt pathway have not been evaluated as possible treatments for PH. All 3 HPIs evaluated in our study inhibited the phosphorylation of Akt and of its downstream effector GSK3. The effects of the HPIs were observed both in vitro and in vivo. The in vitro effects of the 3 HPIs were very similar to those of the selective PI3K inhibitor LY294002, with a marked decrease in cell proliferation contrasting with a small effect on cell apoptosis. The HPI doses required in vitro to block PDGF-induced Akt phosphorylation and PA-SMC proliferation were also similar to those of the specific PI3K inhibitor LY294002 and to those of the HPIs shown in previous studies to inhibit tumor cell growth and to block Akt signaling.12–14 Thus, our finding that the HPIs inhibited Akt suggests mediation of HPI effects by inhibition of GSK3 phosphorylation, ie, GSK3 activation. Accordingly, cell treatment with a GSK3 inhibitor completely abolished the growth-inhibiting effects of amprenavir, nelfinavir, and LY294002 and markedly decreased the inhibitory effect of ritonavir. We also found that each of the 3 HPIs decreased p-Akt in the lungs and pulmonary vessels. To determine whether this effect preceded or was secondary to the changes in pulmonary vascular remodeling, we studied rats after only 1 week of HPI treatment, when only minor changes in pulmonary artery muscularization had occurred. The decreases in p-Akt and p-GSK3 in the pulmonary arteries at this time point, together with the decrease in Ki67-labeled cells in the vascular walls, are consistent with a primary effect of HPI treatment on the Akt signaling pathway, with secondary inhibition of PA-SMC proliferation. At this time point, HPI treatment did not affect the phosphorylation of Erk or MYPT, indicating that HPIs did not interfere with these signaling pathways.
Our results in an experimental model of monocrotaline-induced PH support a protective role of HPIs against HIV-related PH. However, several HPIs, including ritonavir, have been reported to induce endothelial dysfunction in systemic arteries of HIV-infected patients and to diminish endothelium-dependent relaxation and endothelial nitric oxide expression in porcine pulmonary arteries and human pulmonary endothelial cells.24 Because the expression and activity of eNOS are dependent on Akt signaling, the same pharmacological effects of HPIs may lead to 2 apparently opposite vascular effects: one exerted on endothelial cells and responsible for a decrease in eNOS activation with subsequent endothelial dysfunction, and the other exerted on PA-SMCs and responsible for inhibition of cell proliferation. Our results are consistent with this possibility. We found that eNOS protein levels remained unchanged in lungs from monocrotaline-treated rats, in keeping with previous studies showing either unchanged or decreased lung eNOS protein levels in this model.25,26 However, lung p-eNOS, which increased markedly in monocrotaline-treated rats, decreased to basal levels after HPI treatment. Because PA-SMC proliferation is the most prominent abnormality in monocrotaline-induced PH in rats, the antiproliferative effect of HPIs probably played a major role in slowing PH progression in our HPI-treated monocrotaline-injected rats.

Figure 6. Changes in p-eNOS and eNOS levels in lung (A), changes in p-Erk and Erk levels in proximal pulmonary arteries (B), and changes in p-MYPT and MYPT levels in lung (C) in rats injected with saline (controls) or monocrotaline measured 28 days after monocrotaline injection. Treatment with ritonavir, amprenavir, nelfinavir, or vehicle was started at day 21 (n=10 in each group). *P<0.05, **P<0.01, and ***P<0.001 vs values obtained in monocrotaline-injected vehicle-treated rats.
The relevance of our findings to patients with HIV-related PH must be considered with circumspection. Although the HPI doses used in our study animals were chosen on the basis of the plasma levels achieved in humans, the nelfinavir dosage needed to obtain an effect was higher than the dosage used clinically. Thus, our results are consistent with a pharmacological action of HPIs in humans, but other parameters may interfere with HPI effects on the Akt pathway in vivo, including drug pharmacokinetics, concomitant drugs, and side effects, as well as patient characteristics. Prominent side effects of HPI treatment include insulin resistance, diabetes mellitus, and dyslipidemia with increases in low-density lipoprotein cholesterol and triglycerides, body fat accumulation, and lipodystrophy. Previous studies also...

Figure 7. A, Effects of ritonavir, amprenavir, nelfinavir, and LY924002 on PA-SMC proliferation (bar graph) and apoptosis (diamond graph). The cells were starved of FCS for 48 hours and exposed to the HPIs or LY924002 at increasing concentrations during 24 hours. Cellular proliferation was measured with the tetrazolium assay 48 hours after stimulation by 10 ng/mL PDGF-BB. Apoptotic cells were determined at the same time. Values are mean±SEM of 12 values obtained from 4 independent experiments. *P<0.05, **P<0.01, and ***P<0.001 vs values obtained with PDGF alone. The apoptotic cell number increased in response to the 50 μmol/L concentration of ritonavir and nelfinavir (*P<0.05). B, Effects of treatment with amprenavir, ritonavir, nelfinavir, or LY924002 on p-Akt and p-GSK3 protein levels in cultured PA-SMCs stimulated with 10 ng/mL PDGF for 48 hours. Values are mean±SEM of 12 values obtained from 4 independent experiments. *P<0.05, **P<0.01, and ***P<0.001 vs values obtained with PDGF alone.
suggest that diminished Akt activity in response to insulin may lead to insulin resistance. Thus, insulin resistance, a cause of glucose intolerance and dyslipidemia, may be one of the consequences of Akt inhibition by HPIs in HIV-infected patients. Whether the ability of HPIs to inhibit Akt in vivo is useful for treating patients with PH deserves to be investigated.

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Disclosures

None.

References


**CLINICAL PERSPECTIVE**

Pulmonary arterial hypertension (PH) is among the most severe complications of HIV infection. Only limited data are available on the efficacy of PH therapies in HIV-associated PH. Moreover, the potential impact of combination antiretroviral therapy on the progression of HIV-associated PH is still under investigation, and the effects of antiretroviral drugs on pulmonary hemodynamics remain controversial. Here, we show that 3 first-generation HIV protease inhibitors (ritonavir, amprenavir, and nelfinavir) partially protect against the development of hypoxia- or monocrotaline-induced PH in rats. The 3 drugs also partially reversed established PH in monocrotaline-treated rats, an effect associated with diminished pulmonary vascular remodeling due mainly to inhibition of smooth muscle hyperplasia. One suggested mechanism of this growth-inhibiting effect is inhibition of the Akt signaling pathway, which is a downstream target of growth factors such as platelet-derived growth factor and serotonin. These observations are consistent with a pharmacological effect of HIV protease inhibitors in humans because the HIV protease inhibitor doses used in our study animals were chosen on the basis of the plasma levels achieved in humans. Taken together, these results support the ability of HIV protease inhibitors to interfere with pulmonary vascular remodeling by inhibiting Akt phosphorylation and consequently pulmonary artery smooth muscle cell proliferation. Further studies are needed to assess the long-term effects of HIV protease inhibitors on PH progression in HIV-infected patients.
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