Clinical Efficacy of Sildenafil in Patients With Pulmonary Hypertension in Functional Class II or III

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OBJECTIVE: To assess the efficacy of treatment with sildenafil monotherapy in patients with pulmonary hypertension.

PATIENTS AND METHODS: An observational study was undertaken in 11 patients with pulmonary hypertension in functional class II or III who received treatment with sildenafil (150 mg/day). Seven of the patients had inoperable chronic thromboembolic pulmonary hypertension and 4 had pulmonary arterial hypertension. To assess treatment response, the following parameters were assessed during follow-up at 3, 6, and 12 months: exercise tolerance in the 6-minute walk test, change in functional class, and systolic pulmonary arterial pressure measured by echocardiography.

RESULTS: We observed a significant improvement in exercise tolerance, as shown by increased 6-minute walk distance after 3, 6, and 12 months of treatment (increases of 20, 67, and 95 m, respectively). All patients showed an improvement in functional class. The results of echocardiography did not reveal statistically significant differences in systolic pulmonary arterial pressure between baseline and 6 or 12 months of treatment. No significant adverse effects were observed, although sildenafil treatment was suspended in 1 patient due to persistent headache.

CONCLUSIONS: The results of this study confirm that sildenafil is an effective drug for the management of pulmonary arterial hypertension and inoperable chronic thromboembolic pulmonary hypertension both in the short term and medium to long term, and that the drug is well tolerated and shows few side effects.

Key words: *Pulmonary hypertension. Inoperable chronic thromboembolic pulmonary hypertension. Sildenafil. Clinical efficacy.*

Hipertensión pulmonar: eficacia clínica del sildenafilo en clases funcionales II-III

OBJETIVO: Valorar la eficacia del tratamiento con sildenafilo en monoterapia en pacientes con hipertensión pulmonar (HP).

PACIENTES Y MÉTODOS: Se ha realizado un estudio observacional de 11 pacientes con HP en clases funcionales II y III, a quienes se administró tratamiento con sildenafilo (150 mg/día). Siete presentaban HP tromboembólica crónica y no quirúrgica y 4, hipertensión arterial pulmonar. Para valorar la respuesta analizamos a los 3, 6 y 12 meses los siguientes parámetros: tolerancia al esfuerzo mediante el test de la marcha de 6 min, cambio de clase funcional y ecocardiograma para valorar la presión sistólica en la arteria pulmonar.

RESULTADOS: Encontramos una mejoría significativa de la tolerancia al esfuerzo, con un incremento de la distancia caminada en 6 min, a los 3, 6 y 12 meses de tratamiento (+20, +67 y +95 m, respectivamente). Todos los pacientes mejoraron de clase funcional. El seguimiento ecocardiográfico no mostró diferencias estadísticamente significativas entre los valores basal y a los 6 y 12 meses de tratamiento de la presión sistólica en la arteria pulmonar. No observamos efectos adversos significativos, aun cuando se retiró el tratamiento con sildenafilo en un paciente por cefalea persistente.

CONCLUSIONES: Los hallazgos de este estudio confirman que el sildenafilo es un fármaco eficaz para el manejo de la hipertensión arterial pulmonar y la HP tromboembólica crónica y no quirúrgica, tanto a corto como a medio-largo plazo, con buena tolerancia y escasos efectos secundarios.

Palabras clave: *Hipertensión pulmonar. Hipertensión pulmonar tromboembólica crónica y no quirúrgica. Sildenafilo. Eficacia.*

Introduction

Pulmonary hypertension is defined as an increase in mean pulmonary arterial pressure (mPAP) to more than 25 mm Hg at rest or more than 30 mm Hg during exercise, in the absence of left heart disease.[1] The World Health Organization applies a 5-category descriptive classification based on the underlying mechanism.[1] Group I—pulmonary arterial hypertension (PAH)—includes processes of differing etiology but that share common pathophysiologic changes. This group includes idiopathic and familial forms; pulmonary hypertension due to drugs or toxins or secondary to collagen vascular disease, human immunodeficiency virus (HIV) infection, glycogen storage disease, hemoglobinopathies, portal hypertension, congenital systemic-to-pulmonary shunts, pulmonary venoocclusive disease, or pulmonary capillary hemangiomatosis; and persistent pulmonary hypertension of the newborn. Group IV—chronic thromboembolic pulmonary hypertension (CTPH)—includes cases due to thromboembolic conditions affecting either the proximal or distal pulmonary arteries.

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A progressive increase in pulmonary vascular resistance has been observed in patients with disease of the peripheral branches, along with some pathology findings similar to those seen in idiopathic PAH. Thromboendarterectomy is not indicated in this patient group and treatment similar to that used in patients with PAH should be offered.[2]

Irrespective of the cause of pulmonary hypertension, the end result is proliferation and remodeling of the vascular endothelium, leading to right ventricular failure and death.[3]

The principal mechanism implicated in the pathogenesis of the disease is endothelial cell dysfunction, which leads to reduced production of endogenous vasodilators (prostacyclin and nitric oxide) and increased synthesis of vasoconstrictors such as endothelin-1 and thromboxane A_2[3,4] (Figure 1). These findings have led to the development of drugs such as the prostacyclin analogs epoprostenol,[5] iloprost,[6] and treprostinol,[7] which have potent vasodilatory and antiproliferative effects. More recently, selective and nonselective endothelin receptor antagonists such as bosentan[8] and sitaxsentan[9] have been introduced on the market. Although their introduction for use in the treatment of PAH has led to a substantial improvement in patients' quality of life and survival, some of these drugs present administration difficulties and possible side effects, and they are generally expensive.

The therapeutic potential of the nitric oxide pathway has been less explored. Sildenafil, a drug with a potent pulmonary vasodilatory effect,[10] acts through this pathway by inhibiting phosphodiesterase-5 and preventing breakdown of cyclic guanosine monophosphate, leading to an increase in the activity of endogenous nitric oxide. It is an easily administered drug that is well tolerated and displays a good relationship between cost and efficacy, which is beginning to be demonstrated in controlled trials.[11,12]

The aim of this study was to describe the clinical and functional characteristics of patients treated in our hospital for PAH and CTPH in functional class II or III along with their response to sildenafil monotherapy. The patients were studied in a hospital-based pulmonary hypertension clinic and were therefore not affected by the bias or selection associated with enrollment in a clinical trial.[13]

Patients and Methods

Between January 2003 and September 2005, 30 patients with PH attended our clinic. Eleven consecutive patients in functional class II or III were selected for treatment with sildenafil. The drug was prescribed for compassionate use following approval by the Spanish Ministry of Health and Consumer Affairs. The study protocol encompassed clinical assessment and blood analysis, including hematocrit, serology, and analysis of autoimmunity and hypercoagulability. The imaging techniques used were chest radiography, ventilation-perfusion lung scintigraphy, high-resolution computed tomography, computed tomography angiography, magnetic resonance angiography of the chest, and, based on clinical need, pulmonary arteriography. A complete lung function study was also performed, including a 6-minute walk test. Cardiologic assessment included electrocardiography, transthoracic echocardiography, and cardiac catheterization in all cases.

Cardiac catheterization was undertaken under stable conditions in all patients and assessed the following parameters: systolic pulmonary artery pressure (sPAP), diastolic pulmonary arterial pressure (dPAP), mPAP, pulmonary capillary pressure, right atrial pressure, cardiac output, pulmonary vascular resistance, and systemic and pulmonary oxygen saturation.

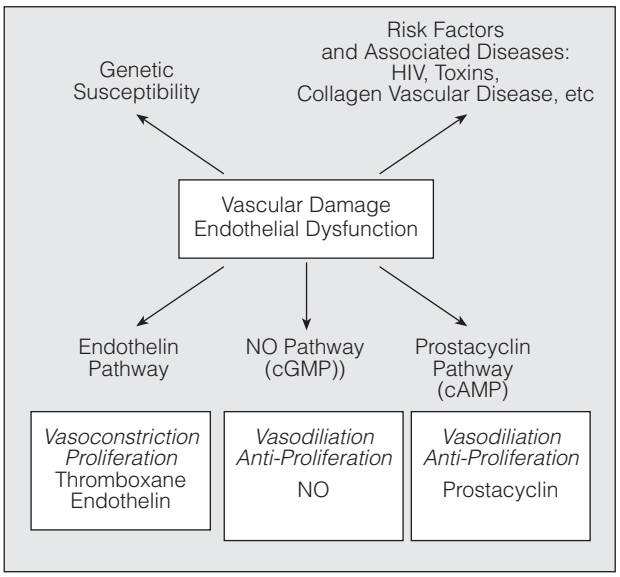

Figure 1. Pathogenic mechanisms involved in pulmonary arterial hypertension. cAMP indicates cyclic adenosine monophosphate; cGMP, cyclic guanosine monophosphate; NO, nitric oxide; HIV, human immunodeficiency virus.

Acute vasodilator challenge was performed with prostacyclin in all cases. Until July 2004, the results of the test were considered positive if a reduction of more than 20% in mPAP was observed,[14] but from that date onwards, we followed international consensus[15] that a positive response corresponded to a reduction of at least 10 mm Hg in mPAP, and that this pressure remained at 40 mm Hg or less, without deterioration of cardiac output.

All patients received anticoagulation therapy with acenocoumarol, 4 received treatment with digitalis, and 7 with furosemide. The patients with CTPH were not considered eligible for thromboendarterectomy because they did not present central thrombi. One patient with CTPH was prescribed continuous home oxygen therapy. Sildenafil was prescribed as monotherapy without addition of other vasodilators. Treatment was initiated during a brief period of hospital admission for blood pressure monitoring and pulse oximetry. Sildenafil was provided at an initial dose of 25 mg every 8 hours, which was increased to 50 mg every 8 hours in the following 48 to 72 hours. Clinical follow-up, blood testing, and a 6-minute walk test were performed every 3 months, while echocardiography was performed every 6 months.

Statistical analysis of the data was carried out with Prism, version 3.0. Results are expressed as medians (interquartile range [IQR]). The Wilcoxon test was used to assess differences between data obtained before and after treatment. Differences were considered statistically significant when the value of *P* was less than .05.

Results

Baseline Characteristics

Of the 11 patients who received treatment, 2 presented idiopathic PAH, 1 had PAH associated with mixed connective tissue disease, 1 PAH associated with HIV infection, and 7 CTPH with involvement of the peripheral

arteries that was not suitable for surgery. One patient with CTPH had a diagnosis of hereditary spherocytosis, for which splenectomy had been performed 35 years earlier. In the remaining patients, no other procoagulant factors were detected during assessment of hypercoagulability (table). The study group contained 5 women and 6 men. The median (IQR) age of the patients was 58 (41.5-70.5) years. The median age of the patients with PAH was 25.5 (12-47) years, lower than the median age of 64 (59-72) years in the group of patients with CTPH.

Prior to initiating the treatment, 5 patients were in functional class II and 6 in class III. Exercise tolerance was assessed with the 6-minute walk test. Under baseline conditions, the patients walked a median distance of 390 (300-460) m.

The magnitude of hypertension calculated using transthoracic echocardiography was severe, with a median systolic pressure of 83 (71-114.5) mm Hg. Hemodynamic data were as follows: sPAP, 80 (45-99) mm Hg; mPAP, 47 (33-55) mm Hg; cardiac index, 3 (1.5-3.5) L/s/m^2; pulmonary vascular resistance, 936 (480-1423) dyn·s/cm^5. The results of acute vasodilator challenge were negative in all cases.

Treatment Response

Following diagnosis and prescription of sildenafil, follow-up was performed in the clinic for all 11 patients over a minimum of 3 months. In 9 patients, follow-up lasted more than 6 months and in 7 patients it was continued for 1 year. Two patients continued to receive treatment for more than 2 years.

Functional class. The 9 patients who received treatment for more than 6 months showed an improvement in functional class, and this improvement was maintained in the 7 patients who reached 1 year of treatment. Of the 2 patients who received 3 months of treatment, 1 improved from functional class III to class II and the other remained stable.

Response to exercise. A continuous improvement in exercise tolerance was observed over the entire follow-up period (Figure 2). At 3 months of treatment, the median distance walked was 410 (284-510) m, an increase of 20 m compared with baseline (*P*=.0002). At 6 months the improvement was 67 m (*P*=.007) and at 12 months it was 95 m (*P*=.015) (Figure 2).

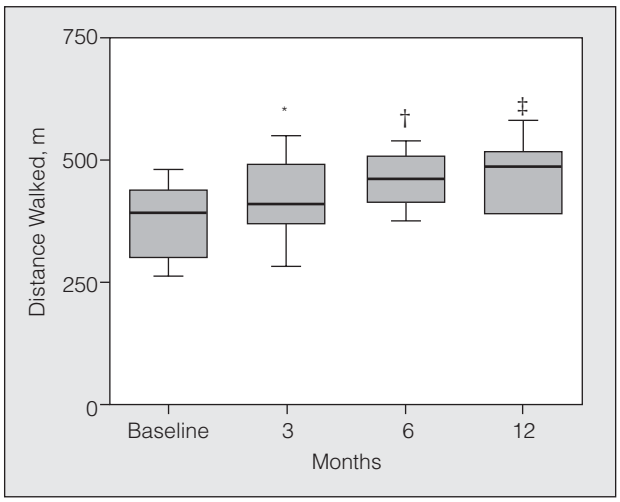

Figure 2. Improvement in the 6-minute walk test. **P*=.002; †*P*=.007; ‡*P*=.015.

Hemodynamic follow-up. Echocardiography was performed at 6-month and 12-month follow-up (Figure 3). The median estimated sPAP was 80 (65-105) mm Hg at 6 months and 67.5 (53-102) mm Hg at 12 months; thus, there was a trend towards decreasing sPAP, but this trend did not achieve statistical significance (*P*=.21 and *P*=.06, respectively).

Tolerance and undesirable effects. None of the patients presented severe side effects. One patient reported nasal congestion, which disappeared within a few weeks of treatment. In another case the treatment was suspended after 4 months due to persistent headache. That patient, who was diagnosed with CTPH with an mPAP of 56 mm Hg, remained stable with no signs of clinical deterioration following suspension of sildenafil treatment.

Baseline Characteristics of Patients Treated With Sildenafil*

Patient	Sex	Age, y	Diagnosis	mPAP, mm Hg	sPAP, mm Hg	sPAP echo mm Hg	NYHA	6MWD, m
1	Female	15	IPAH	54	90	114	III	390
2	Female	12	IPAH	55	75	82	III	380
3	Male	36	PH-HIV	55	85	67	II	440
4	Male	60	CTPH	44	85	109	II	480
5	Male	58	CTPH	56	108	115	II	480
6	Female	55	CTPH	48	115	129	III	300
7	Male	64	CTPH	47	80	83	II	432
8	Female	47	PH-MCTD	35	45	74	III	300
9	Male	71	CTPH	37	60	67	III	285
10	Female	70	CTPH	34	52	68	III	265
11	Male	73	CTPH	70	70	106	II	410

*mPAP indicates mean pulmonary arterial pressure; sPAP, pulmonary arterial systolic pressure measured by right heart catheterization; sPAP echo, pulmonary arterial systolic pressure measured by transthoracic echocardiography; NYHA, New York Heart Association functional class; 6MWD, 6-minute walk distance; IPAH, idiopathic pulmonary arterial hypertension; PH-HIV, pulmonary hypertension associated with HIV infection; CTPH, chronic thromboembolic pulmonary hypertension; PH-MCTD, pulmonary hypertension associated with mixed connective tissue disease.

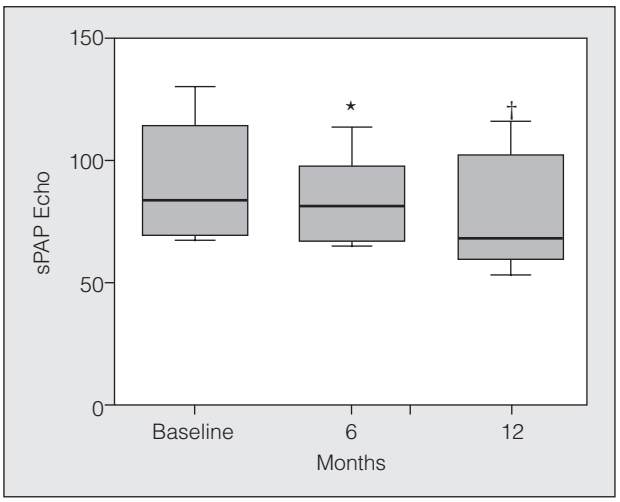

Figure 3. Variation in systolic pulmonary arterial pressure measured by echocardiography (sPAP echo). *P=.2128, †P=.0625.

Discussion

In recent years, advances in our understanding of the pathogenesis of pulmonary hypertension have facilitated the development of new therapeutic targets and led to a radical change in the prognosis of the disease. Treatment algorithms in international guidelines recommend the use of calcium antagonists in those patients who respond to acute vasodilator challenge. Where a negative result is obtained, the guidelines recommend the use of prostanoids, endothelin antagonists, or sildenafil, according to the clinical condition of the patient, the route of administration, the possible side effects, and the cost and availability of the drug.[3,15] Although the guidelines do not establish specific indications for patients in functional class II, it is very likely that early treatment halts the pathogenic processes and that this translates into improved prognosis and survival, even when no specific drug can currently be recommended for this group, as suggested by Badesch et al.[15] On the other hand, it should be remembered that allocating a given patient to functional class II or III is subjective and sometimes inconsistent, and that choice of treatment is influenced by the difficulties of administering the drug and by its possible side effects. Based on these premises, we decided to initiate treatment in our functional class II patients, who corresponded to 45% of the group.

In 2002, when we began to treat patients with sildenafil, only epoprostenol was authorized by the Spanish Ministry of Health and Consumer Affairs. This drug is administered through a central catheter and its use is reserved for patients in an advanced functional class.[5,14] Since none of our patients were in functional class IV, we decided to opt for an orally administered drug, following the treatment algorithms in the international guidelines.

The choice of sildenafil as a first-line treatment was made because preliminary experience with this drug, both in the catheterization laboratory[16] and in isolated cases involving short-term and medium-term treatment,[17] suggested good results, which were later confirmed in clinical trials.

In recent years, extensive experience has been obtained with drug formulations that allow simple administration of vasodilators such as nebulized iloprost or oral bosentan. Sildenafil is a new drug with an efficacy similar to that of bosentan, as shown by Wilkins et al[11] in the only study published to date in which the 2 drugs were compared in the treatment of PAH. These findings, combined with the ease of administration, the limited side effects, and the low cost of the drug, contributed to our election of this treatment option.

The efficacy of sildenafil in the treatment of PAH in functional classes II and III has been demonstrated in a large number of studies, in which the drug has been shown to improve hemodynamic variables, exercise tolerance, and quality of life.[11,12,18-21] However, as is the case with other drugs, data are currently unavailable regarding its impact on survival. We observed a significant clinical improvement in exercise tolerance in the short-term and medium-term following initiation of sildenafil treatment. Thus, our results confirm the findings of clinical trials but in patients from day-to-day clinical practice, who are not subject to the selection requirements associated with large multinational trials. Clearly, we do not have a control group, for ethical reasons and because the focus was on the practical management of the patients, who acted as their own controls.

Treatment with vasodilators has recently been incorporated into the therapeutic options for CTPH involving the peripheral branches, either as a pretreatment prior to surgery[22] or as a chronic treatment in nonsurgical cases.[23,24] Treatment with bosentan and sildenafil leads to marked clinical improvement, with an increase in exercise tolerance and without negative repercussions on gas exchange. This was confirmed in our patients, who showed sustained improvements of this type.

Evaluation over the follow-up period involved assessment of improvement in functional class, exercise tolerance in the 6-minute walk test, and sPAP measured by transthoracic echocardiography. Although we demonstrated a significant and sustained improvement in exercise tolerance, we only observed a nonsignificant tendency towards reduction in the hemodynamic changes assessed by serial echocardiography, as has been reported by other authors.[25] The 6-minute walk test is reproducible, safe, and easy to perform, and also acts as an independent prognostic factor,[26] and therefore complements the results of echocardiography and allows more invasive methods such as right heart catheterization to be avoided. We did not perform right heart catheterization because we do not believe it should be used as a method for follow-up outside of the context of a clinical trial; instead, it should be reserved for specific situations such as changes in treatment due to clinical deterioration or inclusion on the transplant list. In terms of other parameters, classification on the basis of functional class appears not to be very objective and is subject to the interpretations of the patient and observer.

Cardiovascular magnetic resonance imaging has also been proposed for use during follow-up, although as yet with limited experience, since it provides information on the arteries and allows measurement of right ventricular

mass. However, we envisage difficulties in incorporating this technique into health care practice. We should also mention the potential role of biological markers such as brain natriuretic peptide, which, given its usefulness, should be immediately incorporated into routine follow-up.[27]

In terms of the dosage of sildenafil, we administered 150 mg/d in all cases, according to proposals based on reliable experience.[11,19-21,23] Nevertheless, in light of the results of the SUPER trial,[12] there is reason to wonder whether a lower dose (60 mg/d) could be equally effective in patients with less severe disease. Although tolerance of the drug at a dose of 150 mg/d was good and in only 1 case was treatment suspended due to persistent headache, the use of a lower dose would lead to improved tolerance and reduction of treatment costs.

In summary, although the number of patients treated was very limited, the results of this study support sildenafil as an effective drug for the management of PAH and CTPH, both in the short-term and medium to long-term, with good tolerance and few side effects. It remains to be seen what impact these new drugs might have on survival or what advantages might be offered by combination therapy, particularly in patients with an inadequate response to the drug as monotherapy.

REFERENCES

1. Rubin LJ. Introduction. Diagnosis and management of pulmonary arterial hypertension: ACCP evidence-based clinical practice guidelines. Chest. 2004;126:7S-10S.
2. Doyle RL, McCrory D, Channick RN, Simmoneau G, Conte J. Surgical treatments/interventions for pulmonary arterial hypertension. ACCP evidence-based clinical practice guidelines. Chest. 2004;126:63S-71S.
3. Humbert M, Sitbon O, Simonneau G. Treatment of pulmonary arterial hypertension. N Engl J Med. 2004;351:1425-36.
4. Caraballo Fonseca JC, Martínez Blanco CD, Sánchez de León R. Disfunción endotelial en la hipertensión pulmonar. Arch Bronconeumol. 2005;41:389-92.
5. Higenbottam T, Wheeldon D, Wells F, Wallwork J. Long-term treatment of primary pulmonary hypertension with continuous intravenous epoprostenol. Lancet. 1984;1:1046-7.
6. Olschewski H, Simonneau G, Galie N, Higenbottan T, Naeije R, Rubin LJ, et al. Inhaled iloprost for severe pulmonary hypertension. N Engl J Med. 2002;347:322-9.
7. Simonneau G, Barst RJ, Galie N, Naeije R, Rich R, Bourge RC, et al, for the Treprostinil study group. Continuous subcutaneous infusion of treprostinil, a prostacyclin analogue, in patients with pulmonary arterial hypertension: a double-blind randomized controlled trial. Am J Respir Crit Care Med. 2002;165:800-4.
8. Rubin LJ, Badesch DB, Barst RJ, Galie N, Black CM, Keogh A, et al. Bosentan therapy for pulmonary arterial hypertension. N Engl J Med. 2002;346:896-903.
9. Barst RJ, Langleben D, Frost A, Horn EM, Oudiz R, Shapiro S, et al, STRIDE-1 study group. Sitaxsentan therapy for pulmonary arterial hypertension. Am J Respir Crit Care Med. 2004;169:441-7.
10. Reffelman T, Kloner RA. Therapeutic potential of phosphodiesterase 5 inhibition for cardiovascular disease. Circulation. 2003;239: 239-44.
11. Wilkins MR, Paul GA, Strange JW, Tunariu N, Gin-Sing W, Banya W, et al. Sildenafil versus endothelin receptor antagonist for pulmonary hypertension (SERAPH) study. Am J Respir Crit Care Med. 2005;171:1292-7.
12. Galie N, Ghofrani HA, Torbicki A, Barst RJ, Rubin LJ, Badesch D, et al, for the Sildenafil Use in Pulmonary Arterial Hypertension (SUPER) study group. Sildenafil citrate therapy for pulmonary arterial hypertension. N Engl J Med. 2005;353:2148-57.
13. Lexchin J, Bero LA, Djulbegovic B, Clark O. Pharmaceutical industry sponsorship and research outcome and quality: systematic review. BMJ. 2003;326:1-10.
14. Galie N, Torbicki A, Barst R, Dartevelle P, Haworth S, Higenbottam T, et al. ESC guidelines. Guidelines on diagnosis and treatment of pulmonary arterial hypertension. Eur Heart J. 2004;25: 2243-78.
15. Badesch D, Abman SH, Ahearn GS, Barst RJ, McCrory DC, Simonneau G, et al. Medical therapy for pulmonary arterial hypertension. ACCP evidence-based clinical practice guidelines. Chest. 2004;126:S35-S62.
16. Michelackis E, Tymchak W, Lien D, Webster L, Hashimoto K, Archer S. Oral sildenafil is an effective and specific pulmonary vasodilator in patients with pulmonary arterial hypertension: comparison with inhaled nitric oxide. Circulation. 2002;105:2398-403.
17. Prasad S, Wilkinson J, Gatzoulis MA. Sildenafil in primary pulmonary hypertension. N Engl J Med. 2000;343:1342.
18. Michelakis E, Tymchak W, Lien D, Webster L, Hashimoto K, Archer S. Oral sildenafil is an effective and specific pulmonary vasodilator in patients with pulmonary arterial hypertension. Comparison with inhaled nitric oxide. Circulation. 2002;105:2398-403.
19. Mikhail GW, Prasad SK, Li W, Rogers P, Chester AH, Bayne S, et al. Clinical and haemodynamic effects of sildenafil in pulmonary hypertension: acute and mid-term effects. Eur Heart J. 2004; 25:431-6.
20. Humpl T, Reyes T, Holtby HH, Sthephens D, Adatia I. Beneficial effect of oral sildenafil therapy on childhood pulmonary arterial hypertension. Twelve-month clinical trial of a single-drug, open label, pilot study. Circulation. 2005;111:3274-80.
21. Sastry BKS, Narasimhan C, Reddy NK, Raju BS. Clinical efficacy of sildenafil in primary pulmonary hypertension. A randomized, placebo-controlled, double blind, crossover study. J Am Coll Cardiol. 2004;43:1149-53.
22. Nagaya N, Sasaki N, Ando M, Ogino H, Sakamaki F, Kyotani S, et al. Prostacyclin therapy before pulmonary thromboendarterectomy in patients with chronic thromboembolic pulmonary hypertension. Chest. 2003;123:338-43.
23. Ghofrani HA, Schermuly RT, Rose F, Wiedermann R, Kohstall MG, Kreckel A, et al. Sildenafil for long-term treatment of nonoperable chronic thromboembolic pulmonary hypertension. Am J Respir Crit Care Med. 2003;167:1139-41.
24. Hoeper MM, Kramm T, Wilkens H, Schulze C, Schäfers HJ, Welte T, et al. Bosentan therapy for inoperable chronic thromboembolic pulmonary hypertension. Chest. 2005;128:2363-7.
25. Galiè N, Alan L, Hinderliter AL, Torbicki A, Fourme T, Simonneau G, et al. Effects of the oral endothelin-receptor antagonist bosentan on echocardiographic and doppler measures in patients with pulmonary arterial hypertension. J Am Coll Cardiol. 2003; 42:1380-6.
26. Miyamoto S, Nagaya N, Satoh T, Kyotani S, Sakamaki F, Fujita M, et al. Clinical correlates and prognostic significance of six-minute walk test in patients with primary pulmonary hypertension. Comparison with cardiopulmonary exercise testing. Am J Respir Crit Care Med. 2000;161:487-92.
27. Leuchte HH, Holzapfel M, Baumgartner RA, Neurohr C, Vogeser M, Behr J. Characterization of brain natriuretic peptide in long term follow up of primary pulmonary arterial hypertension. Chest. 2005;128:2368-74.