REVIEW ARTICLE

Role Of Iloprost And Bosentan In Pulmonary Arterial Hypertension

Saleh, J A *MBBS, DM*

Galbose Hospital, Jimeta-Yola, Adamawa State

ABSTRACT

Background: *Pulmonary hypertension is a disorder that is perpetually fatal unless a tentative therapy is instituted. It can be described as a syndrome considering its clinical and pathophysiological manifestations. In this disorder, there is an imbalance within the vascular mediators which possibly arises due to pulmonary endothelial cell injury or dysfunction. Pharmacotherapy in PAH is aimed to reverse the imbalance present among the chemical mediators, offer relief to patients from symptoms and prolong their survival. In addition to other supportive measures, iloprost and bosentan form the cornerstone of treatment.*

Iloprost, a vasodilator and stable analogue of prostacyclin, confers great benefit through vasodilation, antiproliferative effects and inhibition of platelet aggregation. Bosentan, an oral non-specific endothelin-receptor antagonist with dual activity on both ETA and ETB receptors, has been shown to improve the patient's quality of life on the overall.

Method: *Review of relevant literature was conducted using manual library search and internet articles. The key words employed were pulmonary hypertension, prostacyclin, endothelin-receptor antagonist, hereditary haemorrhagic telangiectasia, iloprost and bosentan. The National Heart, Lung and Blood Institute website was also used in the course of this review.*

Results: *Several studies were able to outline the haemodynamic advantages of iloprost and bosentan in pulmonary arterial hypertension, as evident by improvement in six-minute walk test of patients treated with these agents.*

Conclusion: *This review was able to outline the pharmacotherapeutic benefits and role played by inhaled iloprost (in addition to its stable nature and minimal adverse effects) and bosentan in the management of PAH. Several studies have shown that these agents improve the patient's quality of life on the overall considering their favourable effect on pulmonary haemodynamics, symptoms reduction and exercise tolerance.*

KEY WORDS: *Pulmonary hypertension, prostacyclin, endothelin-receptor antagonist, hereditary haemorrhagic telangiectasia, iloprost and bosentan*

Date accepted for publication 10th October 2007

Nig J Med 2008; 13 - 19

INTRODUCTION

Pulmonary arterial hypertension (PAH) is an invariably fatal clinical condition characterised by an increase in pulmonary vascular resistance leading to right ventricular failure and, in the absence of definitive treatment, death ensues.[1] It is often described as a syndrome considering its pathophysiological and clinical features; it shortens the life span of patients unless a tentative therapy is instituted.[2] It is defined as a sustained elevation of pulmonary arterial pressure to more than 25mmhg at rest or to more than 30mmhg with exercise with a mean pulmonary-capillary wedge pressure and left ventricular end-diastolic pressure of less than 15mmHg.[3]

PAH was in the past broadly classified into primary (idiopathic) and secondary types until the recent review of this classification by WHO which is based on disease mechanism rather than associated conditions[1]; this classification is presented in table 1.

TABLE 1: WHO revised classification of Pulmonary arterial hypertension[2]

Group I: Pulmonary arterial hypertension (PAH)
- Idiopathic
- Familial
- Related conditionscollagen vascular disease, congenital systemic to pulmonary shunts, portal hypertension, HIV infection, drugs and toxins (eg anorexigens, rapeseed oil, L-tryptophan, methamphetamine and cocaine), other conditions such as thyroid disorders, collagen storage disease, Gaucher's disease, hereditary haemorrhagic telangiectasia, haemoglobinopathies, myeloproliferative disorders, splenectomy
- Associated with considerable capillary involvementsuch as pulmonary veno-occlusive disease, pulmonary capillary haemangiomatosis
- Persistent pulmonary hypertension of the newborn

Group II: Pulmonary venous hypertension with left heart disease
- Left-sided atrial or ventricular heart disease
- Left sided valvular heart disease eg left ventricular systolic and diastolic dysfunction, mitral stenosis and mitral regurgitation

Correspondence to ***Dr. J. A. Saleh,*** *E-mail: jalal.abubakar-saleh03@imperial.ac.uk*

Group III: Pulmonary hypertension associated with hypoxaemia
- Chronic obstructive pulmonary disease
- Interstitial lung disease
- Sleep-disordered breathing
-- Alveolar hypoventilation disorders
- Chronic exposure to high altitude
- Developmental abnormalities

Group IV: Pulmonary hypertension due to chronic thrombotic disease, embolic disease or both
- Thromboembolic obstruction of proximal pulmonary arteries
- Thromboembolic obstruction of distal pulmonary arteries
- Pulmonary embolism (tumour, parasites, foreign material)

Group V: Miscellaneous
Sarcoidosis, pulmonary Langerhans'-cell histiocytosis, lymphangiomatosis, compression of pulmonary vessels (adenopathy, tumor, fibrosing mediastinitis)

However, there is paucity of literature in most developing countries and Nigeria in particular; this could probably be due to under reporting or missed diagnosis of PAH. This review is aimed at sensitizing clinicians to be more vigilant of this fatal but manageable disorder.

PAH is seen in all forms of connective tissue disorders especially localised cutaneous systemic sclerosis (also known as CREST syndrome [calcinosis cutis, Raynaud's phenomenon, oesophageal dysfunction, sclerodactyly, and telangiectasia]), and mixed connective tissue disease, affecting 15-20% of cases.[10] PAH is also seen in sickle cell disease, HIV infection, drug related causes (dexfenfluramine) and environmental (hypoxia).[1]

Considering the prevalence of sickle cell disease and HIV in this environment, PAH as a complication should closely be sought for in these patients. In sickle disease it is estimated that the incidence of pulmonary hypertension varies from 8 to 30 percent thus increasing the risk of death these patients.[4,5] Several studies have shown that in sickle cell anaemia, there is increase level of plasma oxyhaemoglobin due to intracellular haemolysis; this impairs responses to intrinsic and extrinsic nitric oxide. In addition, the intracellular reactive oxygen and that in circulation is increased; this inactivates nitric oxide. Furthermore, the levels of reactive oxygen in circulation and at the intracellular level are increased thus inactivating nitric oxide.[6-8]

It is worthy to note that the advent of HAART (highly active anti-retroviral therapy) has greatly modified the management of HIV but with a rise in complications like PAH (HIV related pulmonary hypertension).[9] The association between HIV and PAH was first reported by Speich et al[11,12] in 1991. The incidence of PAH in HIV was approximately 0.5 percent, a rate on the average of 10 times as high as in the general population. In the report, the HIV patients were haemophiliacs who contracted the disease through blood transfusion after receiving factor VIII. However, this is not only confined to these patients but also other HIV patients who acquired the disease through other routes. The mechanism of PAH in HIV infection still remains obscured notwithstanding the fact that the virus directly infect the vascular endothelial cells leading to HIV associated vasculopathy. Another contributory factor is the deposition of the antigen-antibody complexes in the endothelial lining due to cytokine dysregulation. Although PAH as a complication of HIV is independent of the CD4 cell count, this is directly related to the duration of HIV infection in these patients.[11,12]

In idiopathic PAH, patients should undergo genetic testing and counselling given to relatives. It has been observed that in up to 50% of patients with positive family history of PAH and 25% of those thought to have sporadic idiopathic form of PAH, gene mutations have been identified.[13] The familial PAH is inherited in an autosomal dominant fashion with incomplete penetrance of 10-20%, and age of onset between 1 to 74 years.

PATHOPHYSIOLOGY

Understanding of pathophysiology of PAH has greatly influenced the pharmacotherapy of this clinical condition; the symptoms of seen are as a result of the interplay between vascular mediators which is possibly as a result of pulmonary endothelial cell injury or dysfunction. It is the consequences of this imbalance between vasodilators and vasoconstrictors, growth inhibitors and mitogenic factors, and anthithrombotic and prothrombotic determinants that results in vasoconstriction, proliferation of the smooth muscle and endothelial cells, and thrombosis. Drugs employed in the treatment of this fatal disorder are developed on the basis of this vascular mediator-imbalance.

Despite the fact that pathogenesis of PAH remains obscured, studies have showed that understanding its molecular genetics and cell biology has greatly modified the disease picture.[14,15]

TABLE II:
VASCULAR MEDIATORS IN PULMONARY ARTERIAL HYPERTENSION[59]

NAME BLOOD LEVEL	ACTION
Prostacyclin ↓	vasodilator, platelet antagonist, antiproliferative properties
Thromboxane ↑	vasoconstrictor, platelet agonist
Endothelin-1 ↑	vasoconstrictor
Nitric oxide ↓	vasodilator, inhibits vascular smooth muscle proliferation
Serotonin ↑	vasoconstrictor
VIP ↓	vasodilator, platelet antagonist, inhibits vascular smooth muscle proliferation

↓ = decrease
↑ = increase
VIP= vasoactive intestinal peptide

Table 2 shows the interaction between the various chemical mediators seen in PAH as discussed below.

Prostacyclin and thromboxane A_2: these arachidonic acid metabolites of the vascular cells. In PAH there is a mismatch in the level of these two effectors; prostacyclin (a potent vasodilator with anti-paltelets and anit-proliferative properties) is reduced while there is an increases in thromboxane A_2 (a potent vasoconstrictor and platelet agonist).[16] There is also a decrease in production of prostacyclin synthase in the small and medium-sized pulmonary arteries of these patients.[17] There is also a decrease in the level of prostacyclin metabolite (6-keto-prostacyclin$F_2\alpha$) in the urine of these patients and an increase in that of throboxane A_2 metabolite (thromboxane B_2).

Endothelin-1: Endothelin-1, an endogenous peptide mediator, is a potent vasoconstrictor and smooth muscle mitogen that is over expressed in the plasma and lung tissue of patients with primary pulmonary hypertension.[18-20] It is a pro-inflammatory mediator by virtue of its capacity to enhance the expression of adhesion molecules[21,22] and also induces fibrosis. Its plasma level is increased in pulmonary arterial hypertension[23] further supporting the vascular haemodynamic changes in this fatal disorder. Endothelin-1 mediate its action through two receptors: endothelin-A (ETA) and endothelin-B (ETB) receptors.[22,24] It has been shown that activation of ETB receptors cause the production of nitric oxide and prostacyclin, while activation of ETA receptor leads to vasoconstriction and proliferation of vascular smooth-muscles.[24] The ideal endothelin-receptor antagonist should be specific for ETA.

Vasoactive intestinal peptide: this is a potent systemic vasodilator, platelet antagonists and inhibitor of smooth muscle proliferation.[25,26] Its level is reduced in PAH.[27]

Nitric oxide: synthesis of these potent vasodilator and platelet antagonist is catalysed by nitric oxide synthase. Studies have shown a decrease in the level of the endothelial isoform of this enzyme in patients with pulmonary hypertension.[28,29]

Serotonin (5-hydroxytryptamine): there is an elevated plasma level of this potent vasoconstrictor and promoter of smooth muscle cell hypertrophy and hyperplasia in pulmonary arterial hypertenion.[30,31] It has been shown that patients taking dexfluramine (an appetite suppressant) have an increase chance of developing pulmonary arterial.[32]

Adrenomedullin: considered as a marker PAH and synthesized in the lungs, is a pulmonary vasodilator.[33] It level is elevated in PAH.[34,35]

CLINICAL PRESENTATION

Presentation could be classical or subtle with non-specific symptoms and or signs. Patients may be asymptomatic or have shortness of breath in the early stage. Some may present with exertional dyspnoea, anginal or syncopal attacks. There may be chest pain, fatigue, orthopnoea or paroxysmal nocturnal dyspnoea. Recurrent episodes of acute chest syndrome in sickle cell patients were considered to be the most important risk factor for the development of pulmonary arterial hypertension.[36,37]

However, in the presence of pulmonary congestion as is the case in advanced disease condition, there may be peripheral oedema and abdominal distension. Furthermore, arthralgic and non-specific symptoms in patients with Raynaud's phenomenon are strongly suggestive of PAH. Depending on the underlying condition, murmur could be present with left parasternal heave and loud P_2.[38,39]

INVESTIGATIONS

The aim of investigation in suspected PAH case is to establish the disease and cause sought. Basic

investigations include pulmonary function test, arterial blood gases, chest x-ray, and ECG, serological testing (for connective tissue disease), haemoglobinopathies and HIV screening.

Despite its invasive nature, right heart catheterization still remains the gold standard in clinical practice; it accurately measures resistance of the pulmonary vasculature, directly assesses pulmonary haemodynamics, pulmonary arterial pressure and cardiac output. Nevertheless, echocardiography remains the most useful non-invasive diagnostic tool providing a quantitative estimate of pulmonary arterial pressure among others[40]; it also excludes congenital heart disease. Ventilation-perfusion scanning should be performed to rule it out suspected thromboembolic pulmonary disease[41]; it is a potential curable condition which should not be missed in diagnosis.

TABLE III:
Functional Classification of Pulmonary Arterial Hypertension NYHA

CLASS	DESCRIPTION
I	Pulmonary arterial hypertension without a resulting limitation of physical activity. Ordinary physical activity does not cause undue dyspnoea or fatigue, chest pain, or near syncope
II	Pulmonary arterial hypertension resulting in a slight limitation of physical activity. The patient is comfortable at rest, but ordinary physical activity causes undue dyspnoea or fatigue, chest pain, or near-syncope
III	Pulmonary arterial hypertension resulting in a marked limitation of physical activity. The patient is comfortable at rest, but less than ordinary activity causes undue dyspnoea or fatigue, chest pain, or near-syncope
IV	Pulmonary arterial hypertension resulting in an inability to carry out any physical activity without symptoms. The patient has signs of right heart failure. Dyspnoea, fatigue, or both may be present even at rest, and discomfort is increased by any physical activity

Modified from New York Heart Association classification for heart disease[60].

MANAGEMENT

Despite the fact that there is no definitive cure for PAH, drug treatment has greatly improved the disease picture in the past decade. The aim of pharmacotherapy is to reverse the imbalance present among the chemical mediators, offer relief to patients from symptoms and prolong their survival. In addition to general care, any associated medical condition should be treated and risk factors eliminated. Pharmacotherapy include the use of vasodilators (e.g. prostacyclin analogues, bosentan), oral anticoagulants, supplemental oxygen due to hypoxaemia, diuretics and, in patients with right heart failure, digoxin. Most of the drugs employed in the treatment of PAH exhibit a pleiotropic effect. Other agents that could be employed may have one or more of these properties: vasodilation, anticoagulation, anti-platelet aggregation, anti-inflammatory and vascular re-modelling. The NYHA functional class[58] has been an important end point (table 3) in the clinical trials of pulmonary arterial hypertension. The six-minute walk test has been widely used as a primary end point in clinical trials; this is a test in which the patient walks as far as possible in six minutes. It is employed as a standard exercise test in patients who are incapable of tolerating strenuous exercise testing and is considered as a safe, straight forward test in which the distance covered in six minutes has a strong independent association with mortality among patients with idiopathic pulmonary arterial hypertension.[59,60] This review is centred on iloprost (a prostacyclin analogue) and bosentan (endothelin receptor antagonists) as they form the cornerstone of treatment of PAH

Iloprost: In the 1980s when a search for a pulmonary vasodilator drug started, epoprostenol was developed. This drug, a prostacyclin with anti-platelet aggregation properties became the gold standard in the treatment of PAH; [42] it improves the symptomatology and prognosis of these patients. Several studies have shown a 5-year survival rate in 50-60% of patients receiving treatment with this class of drug.[43-45] The various sites of action of prostacyclin are presented in figure 1.

Prostacyclin given via intravenous route has a limited half life of about three to five minutes in the circulation hence the need for continuous intravenous route.[34] Prostacyclin is a potent vasodilator which has anti-proliferative effects and inhibits platelet aggregation as well. This drug which proved to be very useful in the 80's is not without its limitation; the continuous intravenous administration affects the life style of the patient on ensuring uninterrupted intravenous administration of the agent by means of an external pump or device. In addition to the side effects of the medication, which includes nausea, gastrointestinal upset, rashes and troublesome headache, complications attached to the intravenous use such as local infections and life-threatening sepsis are not uncommon.[34] Furthermore, therapy with this drug is limited by other systemic side effects due to the non-selectivity of the vasodilator response. The tachyphylaxis seen in patients coupled with its long term administration leads to increase in cost of treatment to patients and or government. Iloprost, a stable analogue of prostacyclin, confer great benefit in both primary and secondary types through

vasodilation, anti-proliferative effects and inhibition of platelet aggregation. Studies currently indicate that inhaled aerolised or nebulized iloprost offers potential especially to patients who are not eligible for parenteral therapy.[35,46,47] It became the drug of choice because of its favourable effect on pulmonary haemodynamics, symptom reduction and exercise tolerance.[48]

In a placebo controlled trial of 203 patients by Stacey AB et al, inhaled iloprost significantly improved the combined endpoint of change in New York Heart Association functional class and 10% improvement in 6-minute walk distance.[49]

Charl J et al further showed in a study which involved 126 patients that the inhaled form proves to be safe and effective vasodilator in patients with PAH and right heart dysfunction; the pulmonary haemodynamics was greatly improved evidenced by significant decrease in mean pulmonary artery pressure without altering the mean arterial pressure.[50]

Olschewski H et al further showed the beneficial effect of long-term prostacyclin in pulmonary hypertension with coexisting right heart failure.[51] A study conducted by Hoeper MM et al which involved 31 patients revealed that 24 of them that had treatment with inhaled iloprost for a consecutive period of 12 months had significant improvement in symptoms, haemodynamic and exercise capacity.[52]

- Bosentan: is an oral non-specific endothelin-receptor antagonist with activity on both ETA and ETB receptors.[18-20] Figure 2 shows the endothelin pathway and sites of action of Bosentan.[53] Several studies have underscored the usefulness of bosentan in pulmonary arterial hypertension. This drug is metabolized primarily in the liver and may induce an increase in hepatic enzymes specifically the transaminases; this effect is more pronounced in patients receiving treatment with other endothelin-receptor antagonists, such as ambrisentan and sitaxsentan. Thus constant liver function test in patients on this drug must form part of the management. However, there are no reports of hepatic failure as a result of treatment with bosentan documented so far. This drug is contraindicated in pregnant mothers because of its possible teratogenic effect.

In the bosentan trial, development of abnormal hepatic function appeared to be dose-dependent, a finding that provides a rationale for the approved dose of 125 mg twice daily[54.] Channick et al conducted a study on 33 patients in functional class III of the NYHA who were randomly assigned to receive placebo or bosentan (at a dose of 62.5 mg twice daily for 4 weeks and thereafter at a dose of 125 mg twice daily for at least 12 weeks). It was observed that those on bosentan had a significant improvement in cardiac output, pulmonary artery pressure, vascular resistance, and a mean gain of 76 metres in the six-minute walk test (P=0.02) .[55]

In a double-blind randomized placebo-controlled study [Bosentan Randomized Trial of Endothelin Antagonist Therapy (BREATHE-1)] involving 213 patients lasting for 16 weeks, in NYHA functional class III or IV; two groups were randomly assigned to receive placebo or bosentan (at a dose of 62.5 mg twice daily for 4 weeks and thereafter at a dose of either 125 mg or 250 mg twice daily for at least 12 weeks); it was observed that the mean effect of treatment on the six minute walk test was a gain of 44 m among patients in the overall study population (P<0.001) and 52 m among the patients with primary pulmonary hypertension[54]. The objective of this study was aimed at investigating the effect of bosentan on exercise capacity in a larger number of patients with pulmonary arterial hypertension (including patients in WHO functional class IV [defined as having symptoms at rest]) and to compare two doses (125 and 250 mg twice daily).

In another non-randomized open-label study involving 16 patients in NYHA functional class II-IV with progressive pulmonary arterial hypertension receiving treatment with either beraprost or inhaled iloprost, these patients were placed on 125mg bid bosentan. The study noted that there was a mean gain of 106metres improvement in 6 minutes walking distance after at least 6 months of treatment, an increase in exercise capacity and an improved right ventricular function. There was no dose adjustment or discontinuation of bosentan due to side effects or intolerance.[56]

CONCLUSION

This review was able to show that pulmonary arterial hypertension (PAH) is an invariably fatal clinical condition which is manageable if detected early and accurately. It often presents as a complication or in association with other underlying medical conditions hence the diagnostic puzzle and thus under reported or missed diagnosed. There is a need for physicians to be vigilant of this disorder in clinical practice so that early and accurate diagnosis made, and treatment instituted to improve the patient's quality of life. Iloprost and

Bosentan form the cornerstone of management PAH as studies have shown that they improve pulmonary haemodynamics, symptoms reduction and exercise tolerance enabling the patient run a near normal life.

REFERENCES:

1. Simonneau G, Galie N, Rubin LJ, et al. Clinical classification of pulmonary hypertension. *J Am Coll Cardiol* 2004;43:S512.
2. Barst RJ. Medical therapy in pulmonary hypertension: an overview of treatment and goals. *Clin Chest Med* 2001;22:509-519
3. Gaine SP, Rubin LJ. Primary pulmonary hypertension. *Lancet* 1998;352:719-725.
4. Minter KR, Gladwin MT. Pulmonary complications of sickle cell anemia: a need for increased recognition, treatment, andresearch. *Am J Respir Crit Care Med* 2001;164:2016-2019.
5. Gladwin MT, Sachdev V, Jison ML, et al. Pulmonary hypertension as a risk factor for death in patients with sickle cell disease. *N Engl J Med* 2004;350:886-895.
6. Reiter CD, Wang X, Tanus-Santos JE, et al. Cell-free hemoglobin limits nitric oxide bioavailability in sickle-cell disease. *Nat Med* 2002;8:1383-1389.
7. Klings ES, Farber HW. Role of free radicals in the pathogenesis of acute chest syndrome in sickle cell disease. *Respir Res* 2001;2:280-285.
8. Aslan M, Ryan TM, Adler B, et al. Oxygen radical inhibition of nitric oxide-dependent vascular function in sickle cell disease. *Proc Natl Acad Sci* U S A 2001;98:15215-15220.
9. Speich R, Jenni R, Opravil M, Pfab M, Russi EW. Primary pulmonary hypertension in HIV infection. *Chest* 1991;100:1268-1271.
10. Murata I, Kihara H, Shinohara S, et al. Echocardiographic evaluation of pulmonary arterial hypertension in patients with progressive systemic sclerosis and related syndromes. *Jpn Circ J* 1992;56:983991.
11. Speich R, Jenni R, Opravil M, Pfab M, Russi EW. Primary pulmonary hypertension in HIV infection. *Chest* 1991;100:1268-71.
12. Farber HW. HIV-associated pulmonary hypertension. *AIDS Clin Care* 2001;13:53-59.
13. Lane KB, Machado RD, Pauciulo MW, et al. Heterozygous germline mutations in BMPR2, encoding a TGF-beta receptor, cause familial primary pulmonary hypertension. The international PPH consortium. *Nat Genet* 2000;26:8184.
14. Galie N, Manes A, Branzi A. Protsanoids for pulmonary arterial hypertension. *Am J Respir Med* 2003;2:123-137
15. Mehta S. Drug therapy for pulmonary arterial hypertension: what's on the menu today? *Chest* 2003;124:2045-2049
16. Christman BW, McPherson CD, Newman JH, et al. An imbalance between the excretion of thromboxane and prostacyclin metabolites in pulmonary hypertension. *N Engl J Med* 1992;327:70-75
17. Tuder RM, Cool CD, Geraci MW, et al. Prostacyclin synthase expression is decreased in lungs from patients with severe pulmonary hypertension. *Am J Physiol* 1994;159:1925-32
18. Stelzner TJ, O'Brien RF, Yanagisawa M, et al. Increased lung endothelin-1 production in rats with idiopathic pulmonary hypertension. *Am J Physiol* 1992;262:L614-L620.
19. Allen SW, Chatfield BA, Koppenhafer SA, et al. Circulating immunoreactive endothelin-1 in children with pulmonary hypertension: association with acute hypoxic pulmonary vasoreactivity. *Am Rev Respir Dis* 1993;148:519-22.
20. Giaid A, Yanagisawa M, Langleben D, et al. Expression of endothelin-1 in the lungs of patients with pulmonary hypertension. *N Engl J Med* 1993;328:1732-9.
21. Fagan KA, McMurtry IF, Rodman DM. Role of endothelin-1 in lung disease. *Respir Res* 2001;2:90-101.
22. DiCarlo VS, Chen S-J, Meng QC, et al. ETA-receptor antagonist prevents and reverses chronic hypoxia-induced pulmonary hypertension in rat. *Am J Physiol* 1995;269:L690-L697.
23. Galiè N, Grigioni F, Bacchi-Reggiani L, et al. Relation of endothelin-1 to survival in patients with primary pulmonary hypertension. *Eur J Clin Invest* 1996;26:Suppl 1:A48. abstract.
24. Benigni A, Remuzzi G. Endothelin antagonists. *Lancet* 1999;353:133-8.
25. Petkov V, Mosgoeller W, Ziesche R, et al. Vasoactive intestinal peptide as a new drug for treatment of primary pulmonary hypertension. *J Clin Invest* 2003;111:1339-1346
26. Nicholls MG, Lainchbury JG, Lewis LK, et al. Bioactivity of adrenomedullin and proadrenomedullin N-terminal 20 peptide in man. *Peptides* 2001;22:1745-1752
27. Kakishita M, Nishikimi T, Okano Y, et al. Increased plasma level of adrenomedullin in patients with pulmonary hypertension. *Clin Sci* 1999;96:33-39
28. Lee SL, Wang WW, Lanzillo JJ, et al. Serotonin produces both hyperplasia and hypertrophy of bovine pulmonary artery smooth muscle cells in culture. *Am J Physiol* 1994;266:L46-L52
29. Herve P, Launay JM, Scrobohaci ML, et al. Increased plasma serotonin in primary pulmonary hypertension. *Am J Med* 1995;99:249-254
30. Abenhaim L, Moride Y, Brenot F, et al. Appetite-suppressant drugs and the risk of primary pulmonary hypertension. *N Engl J Med* 1996;335:609-616
31. Hassoun PM, Thappa V, Landman MJ, et al. Endothelin 1 mitogenic activity on pulmonary artery smooth muscle cells and release from hypoxic endothelial cell. *Proc Soc Exp Biol Med* 1992;199:165-170
32. Allen SW, Chatfield BA, Koppenhafer SA, et al. Circulating immunoreactive endothelin-1 in children with pulmonary hypertension: association with acute hypoxic pulmonary vasoreactivity. *Am Rev Respir Dis* 1993;148:519-522.
33. Shimokubo T, Sakata J, Kitamura K, et al. Augmented adrenomedullin concentrations in right ventricle and plasma of experimental pulmonary hypertension. *Life Sci* 1995;57:1771-1779
34. Barst RJ, Rubin LJ, Long WA, et al. A comparison of continuous intravenous poprostenol (prostacyclin) with conventional therapy for primary pulmonary hypertension. *N Engl J Med* 1996;334:296-301
35. Higenbottan T, Butt AY, McMahon A, et al. Long-term intravenous prostaglandin (epoprostenol or iloprost) for treatment of severe pulmonary hypertension. *Heart* 1998;80:151-155
36. Klings ES, Farber HW. Role of free radicals in the pathogenesis of acute chest syndrome in sickle cell disease. *Respir Res* 2001;2:280-285.
37. Platt OS, Brambilla DJ, Rosse WF, et al. Mortality in sickle cell disease life expectancy and risk factors for early death. *N Engl J Med* 1994;330:1639-1644.
38. Gaine SP, Rubin LJ. Primary pulmonary hypertension. *Lancet* 1998;352:719-725.

39. Peacock AJ. Primary pulmonary hypertension. *Thorax* 1999;54:1107-1118. [Erratum, *Thorax* 2000;55:254.]
40. Currie PJ, Seward JB, Chan KL, et al. Continuous wave Doppler determination of right ventricular pressure: a simultaneous Doppler-catheterization study in 127 patients. *J Am Coll Cardiol* 1985;6:750756.
41. Fishman AJ, Moser KM, Fedullo PF. Perfusion lung scans versus pulmonary angiography in evaluation of suspected primary pulmonary hypertension. *Chest* 1983;84:679683.
42. Groves BM, Rubin LJ, Frosolono MF, et al. A comparison of the acute haemodynamic effects of prostacyclin and hydralazine in primary pulmonary hypertension. *Am Heart J* 1985;110:1200-1204
43. Sitborn O, Humbert M, Nunes H, et al. Long-term intravenous epoprostenol infusion in primary pulmonary hypertension: prognostic factors and survival. *J Am Coll Cardiol* 2002;40:780-788
44. McLaughlin VV, Gaine SP, Barst RJ, et al. Efficacy and safety of treprostinil: an epoprostenol analog for primary pulmonary hypertension. *J Cardiovasc Pharmacol* 2003;41:293-299
45. Wensel R, Opitz CF, Anker SD, et al. Assessment pf survival in patients with primary pulmonary hypertension: importance of cardiopulmonary exercise testing. *Circulation* 2002; 106:319-324
46. Olschewski H, Simmoneau G, Galie N, et al. Inhaled iloprost for severe pulmonary hypertension. *N Engl J Med* 2002;347:322-329
47. Olschewski H, Walmrath D, Schermuly R, et al. Aerolised prostacyclin and iloprost in severe pulmonary hypertension. *Ann Intern Med* 1996; 124:820-824
48. Wensel R, Opitz CF, Ewert R, et al. Effects of iloprost inhalation on exercise capacity and ventilatory efficiency in patients with primary pulmonary hypertension. *Circulation* 2000;101:2388-2392
49. Stacey EB, Rebecca HH. Inhaled iloprost in pulmonary arterial hypertension. Formulary forum, published online, June 7 2005.*www.theannals.com*
50. Charl J. De Wet, David G. Affleck, Eric Jacobsohn, et al. Inhaled prostacyclin is safe, effective, and affordable in patients with pulmonary hypertension, right heart dysfunction, and refractory hypoxaemia after cardiothoracic surgery. *J Thorac Cardiovasc Surg* 2004;127:1058-1067
51. Olshewski H, Ghofrani HA, Schehl T, et al. Inhaled iloprost to treat severe pulmonary hypertension. An uncontrolled trial. German PPH Study Group. *Ann Intern Med.* 2000;132:435-443
52. Hoeper MM, Schwarze M, Ehlerding S, et al. Long-term treatment of primary pulmonary hypertension with aerolised iloprost, a prostacyclin analogue. *N Engl J Med* 2000;342:1866-1870
53. Saleh JA. Role of bosentan in pulmonary arterial hypertension. *Nig Med Pract* 2007;51:64-70
54. Rubin LJ, Badesch DB, Barst RJ, et al. Bosentan therapy for pulmonary arterial hypertension. *N Engl J Med* 2002;346:896-903
55. Channick RN, Simonneau G, Sitbon O, et al. Effects of the dual endothelin-receptor antagonist bosentan in patients with pulmonary hypertension: a randomised placebo controlled study. *Lancet* 2001;358:1119-1123.
56. Hans-Jurgen Seyfarth, Hans Pankau, Stefan Hammerschmidt, et al. Bosentan improves exercise tolerance and Tei Index in patients with pulmonary hypertension and prostanoids therapy. *Chest* 2005;128:709713.
57. Farber HW, Loscalzo J. Mechanisms of disease. *N Engl J Med* 2004;351:16551665.
58. British Cardiac Society Guidelines and Medical Practice Committee. Recommendations on the management of pulmonary hypertension in medical practice. *Heart* 2001; 86:Suppl I:I-1I-13.
59. Sitbon O, Humbert M, Nunes H, et al. Long-term intravenous epoprostenol infusion in primary pulmonary hypertension:prognostic factors and survival. *J Am Coll Cardiol* 2002;40:780-788.
60. Miyamoto S, Nagaya N, Satoh T, 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;161:487-492.