Pulmonary and Critical Care Updates

Update on Pulmonary Hypertension 2009

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This article reviews selected studies published in 2009 covering advances in the pulmonary hypertension field. Material covered includes the recent changes in the classification of pulmonary hypertension and the completion of new phase III clinical trials of combination therapy, as well as new studies on the epidemiology, clinical course, and advances in the diagnosis of pulmonary hypertension. We conclude with a survey of studies examining new basic mechanisms of disease and novel therapeutic agents. This summary is necessarily limited and we encourage the reader to obtain the primary manuscripts and related work to examine in more detail.

The year 2009 saw remarkable advances to the pulmonary hypertension field. These studies reported on new basic mechanisms of disease, advances in diagnostics, the discovery of novel therapeutic agents, and the completion of new phase III clinical trials. A PubMed search using the term “pulmonary hypertension” and restricted to the year 2009 yields an astounding 963 new publications. Although this current review is not intended to cover all publications in this rapidly growing field, we will highlight recent changes in the classification and updates on major clinical trial results and review important advances in diagnostic studies and basic science mechanisms.

UPDATED CLASSIFICATION SCHEMA FOR PULMONARY HYPERTENSION FROM THE DANA POINT 4TH WORLD SYMPOSIUM

The updated classification largely maintains the organized and mechanism-based structure of the previous clinical classifications (1). Most of the changes focus on Group 1 (Table 1), pulmonary arterial hypertension (PAH), defined in these meetings as a mean pulmonary artery pressure greater than or equal to 25 mm Hg (approximately 3 SDs above normal) with a pulmonary capillary occlusion pressure of less than or equal to 15 mm Hg. Absolute values for pulmonary vascular resistance and a pressure threshold for exercise were removed. The Group 1 subgroup includes patients who are now defined as hereditary PAH, which includes patients with a family history or patients with idiopathic PAH with germline mutations (e.g., bone morphogenetic protein receptor-2, activin receptor-like kinase type 1, and endoglin). Two other notable changes were made: first, schistosomiasis and chronic hemolytic anemia were

added as separate entities in the subgroup of PAH; second, pulmonary venoocclusive disease and pulmonary capillary hemangiomatosis were added as a separate subgroup of Group 1, now called Group 1'. The latter change was made to reflect the similar pathogenesis between these diseases and the Group 1 plexogenic vasculopathies, while at the same time highlighting their unique features. We refer the reader to more recent guidelines for the diagnosis and treatment of pulmonary hypertension (2, 3).

UPDATE ON DIAGNOSIS AND DEFINITIONS OF PAH BY RIGHT HEART CATHETERIZATION, EXERCISE, AND DOPPLER ECHOCARDIOGRAPHY

Effect of Borderline Mean Pulmonary Artery Pressure Elevations on Functional Outcome

Although PAH has been defined as a mean pulmonary artery pressure (MPAP) greater than or equal to 25 mm Hg (and 30 mm Hg during exercise), this resting value is approximately 3 SDs above the normal resting value (4). Kovacs and colleagues reviewed published data on 1,187 individuals from 47 studies in 13 countries (4). This analysis indicated that MPAP at rest was 14.0 ± 3.3 mm Hg and was independent of sex and ethnicity and slightly influenced by posture (supine, 14.0 ± 3.3 mm Hg; upright, 13.6 ± 3.1 mm Hg) and age (<30 yr, 12.8 ± 3.1 mm Hg; 30–50 yr, 12.9 ± 3.0 mm Hg; ≥ 50 yr, 14.7 ± 4.0 mm Hg). During mild exercise, MPAP was 19.4 ± 4.8 mm Hg in subjects younger than 50 years of age compared with 29.4 ± 8.4 mm Hg in subjects 50 years or older ($P < 0.001$). This indicates that MPAP rarely exceeds 20 mm Hg at rest and exercise MPAP frequently exceeds 30 mm Hg, especially in elderly individuals. These studies provide information on normal rest and exercise pressures and also suggest more study is required in aging subjects to determine if the observed increases in the elderly reflect age-related vascular stiffness, the development of left ventricular diastolic dysfunction, or other occult cardiopulmonary disease manifestations.

Do borderline increases in MPAP influence clinical outcomes in patients at risk for the development of PAH? Because previous studies of patients with chronic obstructive pulmonary disease (COPD) and lung fibrosis suggested that a resting MPAP of greater than 17 mm Hg may be associated with adverse events and reduced survival (5), Kovacs and colleagues evaluated this break point in patients with scleroderma. They enrolled patients with a systolic pulmonary artery pressure of greater than or equal to 40 mm Hg during exercise, estimated by Doppler echocardiography, or a peak VO_2 less than 75% of predicted (6). These patients underwent right heart catheterization and were evaluated in the study only if their MPAP was less than 25 mm Hg. They analyzed the cohort based on having an MPAP above and below the median value of 17 mm Hg. Remarkably, the patients in the group with a value greater than or equal to 17 mm Hg (mean MPAP for group of 20 mm Hg versus 14 mm Hg in the < 17 mm Hg group) had a lower max-

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TABLE 1. UPDATED CLINICAL CLASSIFICATION OF PULMONARY HYPERTENSION FROM DANA POINT, 2008 (1)

1. Pulmonary arterial hypertension (PAH)
1.1. Idiopathic PAH
1.2. Heritable
1.2.1. BMPR2
1.2.2. ALK1, endoglin (with or without hereditary hemorrhagic telangiectasia)
1.2.3. Unknown
1.3. Drug- and toxin-induced
1.4. Associated with
1.4.1. Connective tissue diseases
1.4.2. HIV Infection
1.4.3. Portal hypertension
1.4.4. Congenital heart diseases
1.4.5. Schistosomiasis
1.4.6. Chronic hemolytic anemia
1.5. Persistent pulmonary hypertension of the newborn
1'. Pulmonary venoocclusive disease (PVOD) and/or pulmonary capillary hemangiomatosis (PCH)
2. Pulmonary hypertension owing to left heart disease
2.1. Systolic dysfunction
2.2. Diastolic dysfunction
2.3. Valvular disease
3. Pulmonary hypertension owing to lung diseases and/or hypoxia
3.1. Chronic obstructive pulmonary disease
3.2. Interstitial lung disease
3.3. Other pulmonary diseases with mixed restrictive and obstructive pattern
3.4. Sleep-disordered breathing
3.5. Alveolar hypoventilation disorders
3.6. Chronic exposure to high altitude
3.7. Developmental abnormalities
4. Chronic thromboembolic pulmonary hypertension (CTEPH)
5. Pulmonary hypertension with unclear multifactorial mechanisms
5.1. Hematologic disorders: myeloproliferative disorders, splenectomy
5.2. Systemic disorders: sarcoidosis, pulmonary Langerhans cell histiocytosis; lymphangioleiomyomatosis, neurofibromatosis, vasculitis
5.3. Metabolic disorders: glycogen storage disease, Gaucher disease, thyroid disorders
5.4. Others: tumoral obstruction, fibrosing mediastinitis, chronic renal failure on dialysis

imal work rate, lower $\dot{V}O_2$, and substantially lower 6-minute walk distance. This was despite a mean pulmonary vascular resistance (PVR) of only 168 ± 47 dyn·s·cm⁻⁵. An analysis of patients above and below the median MPAP values at submaximal exercise also predicted lower exercise capacity. These data suggest that in certain populations the definitions of PAH may need to be refined to capture patients with symptomatic disease. Such findings may be of particular relevance in diseases associated with significant associated organ dysfunction that further compromises cardiopulmonary function, such as advanced lung diseases (COPD and idiopathic pulmonary fibrosis [IPF]) and sickle cell disease. However, the extension of diagnosis and therapy to lower PVR cohorts must be established in the setting of robust clinical trials with meaningful endpoints.

Accuracy of Doppler Echocardiography in the Diagnosis of PAH

The Doppler-echocardiographic assessment of the regurgitant jet velocity across the tricuspid valve can be used to estimate the right ventricular and pulmonary artery systolic pressures using a modified Bernoulli equation ($4V^2$, where V = velocity of the tricuspid regurgitant jet). It is necessary to add an estimate of the central venous pressure, which can be determined by degree of collapse of the inferior vena cava during inspiration. A value for the tricuspid regurgitant jet velocity greater than or equal to 2.5 m/sec is approximately 2 SDs above normal (for patients <

40 yr of age the normal reference mean Doppler echocardiographic estimated pulmonary artery systolic pressure is 27.5 ± 4.2 with a 95% confidence interval [CI] of 19.3–35.5 mm Hg) (7). In practice, a more traditional definition of pulmonary hypertension would be a tricuspid regurgitant jet velocity of greater than or equal to 3.0 m/sec. However, in some populations, such as patients with sickle cell disease, the risk of death associated with high pulmonary artery systolic pressures increases linearly with increases in TRV. Even values between 2.5 and 2.9 m/sec are associated with a high risk of death with an odds ratio for death of 4.4 (95% CI, 1.6–12.2; $P < 0.001$); a TRV greater than or equal to 3 m/sec is associated with an odds ratio for death of 10.6 (95% CI, 3.3–33.6; $P < 0.001$) (8). Moreover, stress Doppler echocardiography was used to identify patients at risk of developing pulmonary hypertension among family members of patients with familial PAH (9). These findings in populations must be balanced by the high rates of false-positive screening tests that may lead to aggressive testing and inappropriate therapy in individual patients.

Although there have been many studies evaluating the accuracy of Doppler echocardiography, this question was carefully evaluated in a recent study by Fischer and colleagues (10). They evaluated 65 subjects with various forms of pulmonary hypertension who had a Doppler echocardiogram performed within 1 hour of a right heart catheterization. They reported that Doppler echocardiography was inaccurate (defined as being greater than ± 10 mm Hg of the invasive measurement) in 48% of cases. Overestimation and underestimation of pulmonary artery systolic pressure by Doppler echocardiography occurred in 16 and 15 cases, respectively. Similar problems were observed in the estimated cardiac output. They conclude that echocardiography remains an invaluable screening tool but must include assessments of multiple cardiac parameters and functional studies and must be confirmed by right heart catheterization. They note that particular caution should be exercised in estimating pulmonary artery pressures when the tricuspid jet quality is low, as serious pressure underestimations can occur, leading to missed or delayed diagnosis. Although the Doppler echocardiogram has important limitations, it remains the most useful study for population-based screening and population studies of PAH prevalence and associated risk and was recently also used in children with sickle cell disease (11). However, it should be complemented in such studies with other echocardiographic indices of right heart function and pulmonary pressure, such as the tricuspid annular plane systolic excursion (TAPSE) and pulmonary artery acceleration time, and with functional parameters, such as 6-minute walk distance, and other biomarkers, such as the levels of brain natriuretic peptide.

UPDATE ON MAJOR CLINICAL TRIAL RESULTS

A number of phase II–III pivotal clinical trials were completed in 2009 that help us understand the impact of new therapies, long-term treatment, or combined therapies:

Combination Therapy

Although one of these studies was completed late in 2008, it is of value to highlight as it is one of the major studies suggesting that combination therapy improves exercise performance (12). In this 16-week trial, oral sildenafil titrated to 80 mg three times daily was added to long-term intravenous epoprostenol in patients with pulmonary arterial hypertension. A placebo-adjusted increase of 28.8 m (95% CI, 13.9–43.8 m) in the 6-minute walk distance was observed in patients in the sildenafil group and the addition of sildenafil decreased MPAP by 3.8 mm Hg (CI, –5.6 to –2.1 mm Hg) and increased cardiac output by 0.9 L/min (95% CI, 0.5–1.2 L/min).

A phase III trial of tadalafil, a long-acting phosphodiesterase 5 inhibitor, that increases the levels of cGMP to increase pulmonary vasodilation, was completed in 2009 and provided evidence of efficacy for this agent and an evaluation of combination therapy with an endothelin receptor blocker (13). In this trial, 405 patients who were either on no background therapy or taking the endothelin receptor antagonist bosentan were randomized to placebo or tadalafil at a dose of 2.5, 10, 20, or 40 mg orally once daily. Tadalafil was observed to increase the distance walked in 6 minutes (placebo-adjusted treatment effect of 33 m; 95% CI, 15–50 m) in a dose-dependent manner but only the 40-mg dose achieved the prespecified level of statistical significance. Interestingly, the effect was greater in patients on no background (44 m; 95% CI, 20–69 m) compared with those on bosentan (23 m; 95% CI, –2 to 48 m).

Long-term Effects of Endothelin A Receptor Blockade

The results of the 2-year follow-up study of ambrisentan, which is an oral, once-daily selective endothelin type A receptor antagonist, revealed that after 2 years of ambrisentan exposure, the mean change from baseline in 6-minute walk distance was improved for the 5-mg and 10-mg doses (+23 m and 28 m, respectively) groups. Estimates of survival and freedom from clinical worsening for the combined dose group were 94% and 83%, respectively, at 1 year and 88% and 72%, respectively, at 2 years. From a safety standpoint, the yearly risk of aminotransferase abnormalities (greater than three times the upper limit of normal) was 2% per year (14). These safety data were also corroborated by data from a study in which the course of patients who received ambrisentan, after previously developing hepatotoxicity with either bosentan or sitaxsentan was described (15). The recurrence rate of liver function tests three times above the upper limits of normal did not occur in this patient population with long-term ambrisentan therapy.

UPDATE ON SPECIAL CLINICAL CONDITIONS ASSOCIATED WITH PULMONARY HYPERTENSION

Connective Tissue Disease–associated PAH

Traditionally it is believed that patients with PAH in association with connective tissue disease (CTD-PAH) have a worse prognosis than other subgroups. One-year survival rates for patients with systemic sclerosis–associated PAH (SSc-PAH) of 45% have been published from the era before specific drugs to treat PAH became available. In all randomized controlled trials in the field of PAH, the CTD subgroup showed less functional improvement and faster progression of the disease when compared with the patients with idiopathic PAH. In comparison to historical data, those outcomes appeared to be better than expected. A number of studies in 2009 have characterized patients with CTD-PAH (16, 17) as well as evaluating new candidate molecules for potential targeted therapy of this patient population (18). Condliffe and colleagues have now provided important new data from a reasonably sized patient group from the United Kingdom that might challenge the previous survival estimates (19). They interrogated the U.K. National registry for all incident cases of CTD-PAH diagnosed consecutively between January 2001 and June 2006 and found 429 patients with CTD-PAH (73% SSc-PAH) with catheter-confirmed pulmonary hypertension. Based on the registry data a prevalence of 2.93 per 1 million diagnosed SSc-PAH cases was calculated.

If patients had isolated SSc-PAH their 1- and 3-year survival rates were better (78% and 47%) as compared with those with

respiratory disease–associated SSc-PAH (3-yr survival, 28%; $P < 0.005$). The authors additionally found that age, sex, mixed venous oxygen saturation, and World Health Organization functional class were independent predictors of survival in isolated SSc-PAH. The group of patients with systemic lupus erythematosus had a significantly better 3-year survival rate (75%) than patients with SSc-PAH ($P < 0.01$). The authors thus conclude that survival of patients with SSc-PAH in the modern treatment era is better than in historical series. Previous observations that concomitant respiratory disease is a strong negative predictor of survival were confirmed. Most importantly, these data remind us that comparison of the therapeutic efficacy of new drugs with historical controls is not appropriate.

Portopulmonary Hypertension

According to the current clinical classification (1), portopulmonary hypertension represents a subgroup of World Health Organization category 1 PAH. Little is known about which factors predispose a subset of patients with liver cirrhosis and portal hypertension to develop PAH. Roberts and colleagues aimed to identify genetic risk factors for portopulmonary hypertension in patients with advanced liver disease (20). In a multicenter case-control study of patients with portal hypertension they matched 31 patients who fulfilled hemodynamic criteria for PAH and 104 controls without PAH. Genotyping 1,079 common single nucleotide polymorphisms (SNPs) in 93 candidate genes in each patient revealed 29 SNPs in 15 candidate genes that were associated with the risk of portopulmonary hypertension ($P < 0.05$). Multiple SNPs in the genes coding for estrogen receptor 1, aromatase, phosphodiesterase 5, angiotensin 1, and calcium-binding protein A4 were associated with the risk of portopulmonary hypertension. Interestingly, the authors were able to associate one of the aromatase SNPs with differences in plasma estradiol levels. They concluded that genetic variation in estrogen signaling and cell growth regulators is associated with the risk of developing portopulmonary hypertension.

High-Altitude Pulmonary Hypertension and Edema

Acute hypoxia induces pulmonary vasoconstriction and chronic hypoxia promotes pulmonary vascular remodeling. The impact of pulmonary hypertension on the course of high-altitude pulmonary edema (HAPE), however, is a matter of ongoing controversy; inflammation, lung vascular endothelial leakage, and myocardial dysfunction have been proposed as major pathogenic features. In addition to antiinflammatory agents, a number of vasodilators have been investigated for their ability to prevent or treat HAPE. Pulmonary hypertension at high altitudes potentially contributes to reduced exercise ability as a consequence of increased right heart load. This notion was supported by studies showing that phosphodiesterase 5 inhibition could improve exercise ability at high altitudes (21, 22). Fischler and colleagues have now evaluated the impact of either dexamethasone, tadalafil, or placebo on exercise capacity in subjects prone to develop HAPE (23). Twenty-three subjects with a previous history of HAPE were randomized to dexamethasone at 8 mg twice a day, tadalafil at 10 mg twice a day, or placebo (double-blinded), starting the day before ascent to an altitude of 4,559 m. Dexamethasone improved maximum oxygen uptake and oxygen kinetics and reduced the ventilatory equivalent for CO₂. Echocardiography at low-intensity exercise (40% of peak power) revealed that dexamethasone was most effective in lowering the pulmonary artery pressure compared with placebo or tadalafil. The authors therefore conclude that corticosteroids, but not phosphodiesterase-5 inhibition, partially prevented the limitation of exercise capacity in subjects with intense hypoxic pulmonary

vasoconstriction at high altitude, supportive for a predominant role of inflammation and vascular/epithelial leakage in HAPE. This interpretation should be balanced by appreciation that the dose of Tadalafil used was lower than the effective doses for PAH discussed earlier in this update.

Pulmonary Embolism and Chronic Thromboembolic Pulmonary Hypertension

The long-term outcome for patients who survive acute pulmonary embolism is dependent on multiple factors. In a prospective study, Klok and coworkers followed 308 patients with unprovoked and 558 patients with provoked pulmonary embolism, defined as an embolism occurring in the absence (unprovoked) and presence (provoked) of established risk factors or predisposing illnesses. They also included 334 control patients without pulmonary embolism and followed the three groups for a median period of 3.3 years (24). They found that patients with unprovoked pulmonary embolism had lower overall risk for mortality than patients with provoked pulmonary embolism, (adjusted hazard ratio [HR] 0.59; 95% CI, 0.43–0.82), but higher risk for non-malignancy-related mortality (HR, 1.8; 95% CI, 1.3–2.5), recurrent venous thromboembolism (HR, 2.1; 95% CI, 1.3–3.1), cancer (HR, 4.4; 95% CI, 2.0–10), cardiovascular events (HR, 2.6; 95% CI, 1.5–3.8), and chronic thromboembolic pulmonary hypertension (CTEPH) (1.5% vs. 0%). Interestingly, the risk of composite major clinical complications after pulmonary embolism was similar in both groups. The authors find that 50% of patients surviving acute pulmonary embolism experience serious adverse events within 4 years (compared with 16% in the control group), suggesting that patients with pulmonary embolism should receive intensive follow-up, screening, and therapy regardless of predisposing illness and risk factors.

Although the treatment of choice for patients with CTEPH remains surgical pulmonary thromboendarterectomy, the aforementioned risk stratification should be taken into account. Before pulmonary thromboendarterectomy the distinction must be made between proximal disease, with lesions that are surgically accessible, and distal disease, mainly characterized by peripheral lesions and small-vessel remodeling. Although an intriguing percentage of patients with CTEPH sustain pulmonary vasoreactivity (25), supporting the notion that this is not simply an obstructive disease, medical treatment with PAH-specific drugs is frequently used in the absence of randomized placebo-controlled clinical trials. Little was known so far about the usefulness of treating operable patients with PAH drugs before surgery. Jensen and colleagues from the center in San Diego—by far the most experienced group in this field—have now provided important data suggesting that medical pretreatment of patients with operable CTEPH has no beneficial impact on postsurgical outcomes and hemodynamics (26).

UPDATE ON THE IMPACT OF THE NEW LUNG TRANSPLANTATION ALLOCATION SCORING SYSTEM ON PATIENTS LISTED WITH PULMONARY ARTERIAL HYPERTENSION

Chen and colleagues reviewed the effects of changes to the lung allocation system in 2005 on the likelihood of patients with PAH receiving a lung while on the waiting list (27). In 2005, the system was changed to direct organs to patients with a high Lung Allocation Score (LAS) rather than being based on the time a patient was listed. The score is derived by predictive models that balance the risk of death while on the waiting list against the predicted post-transplant survival at 1 year, using factors such as underlying disease, functional status, exercise capacity, hemodynamics, and the need for oxygen or mechan-

ical ventilation. This change was intended to direct organs to the most ill patients, such as those with IPF, to reduce the mortality rate of patients on the waiting list. In the Chen study, 7,952 patients listed for lung transplantation between 2002 and 2008 were evaluated and the waiting list mortality and post-transplant mortality compared before and after the implementation of the LAS. They found that with the new system the probability of receiving a lung while on the waiting list actually increased for all diseases, including PAH, and the waiting list mortality decreased for every diagnosis. There was no effect on post-transplant outcomes, indicating that the selection of more ill patients to transplant did not adversely affect outcomes. Of potential concern, using the LAS system patients with PAH were less likely to be transplanted than patients with IPF or cystic fibrosis and were at greater risk of death on the waiting list than patients with COPD or cystic fibrosis. These findings strongly suggest that some modification of the scoring for patients with PAH is necessary, such as integration of information on right ventricular function.

UPDATE ON NEW MECHANISMS, THERAPEUTIC TARGETS, AND CANDIDATE DRUGS

Compensatory Lung Parenchymal Remodeling Associated with Chronic Pulmonary Venous Hypertension

Chronic increases in pulmonary microvascular pressures in the setting of congestive heart failure lead to compensatory protective remodeling of the lung parenchyma and pulmonary circulation. One such compensatory mechanism involves a thickening of the alveolocapillary barrier that decreases the capillary filtration coefficient and reduces alveolar fluid accumulation. Little is known, however, about early protective compensatory mechanisms that maintain gas exchange and help preserve lung mechanics under conditions of chronic heart failure. Dixon and colleagues examined lung function in a rat model of chronic heart failure (28). In keeping with prior studies, they found an increase in dry lung weights due to increased insoluble protein, lipid, and cellular infiltrate, but no pulmonary edema. Although the lungs were stiffer, the measured surface tension in the alveoli was reduced, mediated by increases in both the surfactant content and alveolar type II cell numbers. This finding was proposed as an additional compensatory mechanism that reduces the work of breathing in the setting of chronic heart failure.

Peroxisome Proliferator-activated Receptor γ and PAH

Peroxisome proliferator-activated receptor γ (PPAR γ) expression is reduced in animal models and in patients with PAH and the synthetic thiazolidinedione (TZD) PPAR γ agonists (e.g., rosiglitazone) attenuate the development and progression of PAH (29). The mechanisms accounting for the attenuation of the initiation and progression of PAH by PPAR γ remain unclear. Inasmuch as endothelial dysfunction plays a vital role in both systemic and pulmonary vascular diseases, it is notable that PPAR γ agonists increase NO production in endothelial cells by post-translational endothelial nitric oxide synthase (eNOS) modifications and not by increased eNOS expression. PPAR γ activation is proposed to retard the initiation and development of PAH by mechanisms all centered on increased NO bioavailability, including (1) inhibition of proinflammatory signaling reactions, (2) decreased SMC and endothelial cell migration and proliferation, and (3) decreased production of reactive species via reduction in the expression and activity of NADPH oxidase (Nox4). The therapeutic potential of PPAR γ agonists also reveals promise in animal models of PAH. In 2009, Nisbet and colleagues explored this pathway in the chronic hypoxic mouse PAH model (30). They found that treatment with rosiglitazone prevented the development of

pulmonary hypertension at 3 weeks, reversed established pulmonary hypertension at 5 weeks, and from a mechanistic standpoint reduced NADPH oxidase (Nox4) expression, superoxide production, and platelet-derived growth factor (PDGF) receptor β activation.

Although these studies are promising, clinical complications of TZDs are emerging for the treatment of diabetes with dual PPAR γ and PPAR α agonists (e.g., muraglitazar), intended to offset the adverse fluid retention and weight gain of “straight” PPAR γ agonists such as rosiglitazone. Clinical use of muraglitazar has been halted following the recent report that it may result in increased adverse myocardial events and death. A metaanalysis of treatment trials of rosiglitazone, when compared with other therapies for type 2 diabetes or placebo, showed a modest increase in the risk of myocardial infarction and a borderline significant finding for increased death from cardiovascular causes ($P = 0.06$) (31). The net clinical benefits of TZDs are thus presently a source of intense debate and should not be used in PAH until a properly designed clinical trial justifies their use.

Inhaled Nitrite Therapy for PAH

Because PAH is associated with a decreased bioavailability of NO, targeting of the NO pathway has been proposed as potential therapy. Recent studies also indicate that NO formed from eNOS is oxidized to the anion (salt) nitrite (NO_2^-) by oxygen or plasma ceruloplasmin. Nitrite transport in blood provides an endocrine form of NO that can be shuttled from the lungs to the periphery, while limiting the exposure of authentic NO to the scavenging red cell environment. Then during the rapid hemoglobin deoxygenation from artery to vein, nitrite is reduced back to NO via reactions with hemoglobin, myoglobin, and other heme-containing enzymes, such as xanthine oxidoreductase (32). Once formed, nitrite is a potent vasodilator, potentially regulating basal blood flow and hypoxic vasodilation. Additionally, nitrite limits hypoxic pulmonary vasoconstriction in a hypoxia- and pH-dependent fashion. Given the central role of NO in vasoregulation, the association of decreased NO bioavailability in PAH and recently emerging signaling actions of nitrite, Zuckerbraun and colleagues (33) evaluated a new NO-directed therapy, inhaled sodium nitrite (NO_2^-). The authors delivered nebulized nitrite to the hypoxic mouse PAH model and the monocrotaline rat model and found that low doses delivered once or three times a week both prevented and reversed the development of PAH. They further described that nitrite was converted to NO via the actions of pulmonary xanthine oxidoreductase, leading to downstream augmentation of the cell cycle inhibitor p21.

Stimulators of the Soluble Guanylate Cyclase

Although PDE5 inhibitors are a mainstay of PAH therapy (34), the mechanism of action is dependent on a functional upstream signaling cascade (i.e., it requires an intact NO–soluble guanylyl cyclase [sGC]–cGMP pathway) (35). On the other hand it is well known that this signaling axis is impaired on many levels in pulmonary and systemic vascular disorders. It could therefore be advantageous to tackle this important vasodilative/antiproliferative pathway by direct stimulators or activators of the enzyme sGC downstream of NO production. In a proof of concept study the acute hemodynamic effects of riociguat, a novel first-in-class stimulator of the sGC, showed good efficacy in lowering PVR in patients with moderate to severe precapillary pulmonary hypertension (36). Of note, the reductions in PVR were significantly greater with either 1 mg or 2.5 mg of riociguat when compared with inhaled NO in the same patient groups. Although systemic vascular resistance was

reduced in the same range as the pulmonary vascular resistance, patients did not complain about symptoms associated with hypotension. This is most likely explained by an adequate increase of cardiac output, which offset clinically meaningful reductions in systemic blood pressure. The results of a phase II long-term treatment study with the same compound have been presented in abstract form and two phase III randomized controlled trials are currently underway to assess the efficacy of this compound in patients with PAH and CTEPH.

Protective Role of Angiotensin-converting Enzyme-II in Pulmonary Hypertension

Previous publications suggested that inhibitors of the angiotensin-converting enzyme (ACE) I could alleviate pulmonary hypertension and reduce right heart hypertrophy (37). However, the bulk of experimental and preclinical data were derived from preventive rather than therapeutic interventions in animal models of PAH, and have so far not been supported by clinical data. In addition, ACE inhibitors are known to possess anti-hypertrophic properties on the myocardium. In patients with severe precapillary pulmonary hypertension, in whom adaptive right heart hypertrophy is an essential mechanism to cope with the increased afterload, pharmacologic regression of right ventricular hypertrophy could thus be potentially detrimental. Ferreira and colleagues have now provided intriguing new data that the ACE II is up-regulated in both patient tissue as well as material harvested from pulmonary hypertensive animals, and that treatment of monocrotaline rats with an activator of ACE II (XNT [1-([2-dimethylamino] ethylamino)-4-(hydroxymethyl)-7-([4-methylphenyl] sulfonyl oxy)-9Hxanthene-9-one]) could prevent the development of pulmonary hypertension and lung vascular remodeling (38). Despite the limitations of a preventive treatment approach the data still warrant further investigation regarding a potential clinical usefulness, provided clinically suitable ACE II activators become available.

Endothelial Progenitor Cells

Controversy exists about the levels of endogenous endothelial progenitor cells in PAH and whether they may or may not be of benefit if expanded *ex vivo* and subsequently applied therapeutically. Toshner and colleagues published data that imply that endothelial progenitor cells contribute to vascular remodeling in PAH (39). They found an up-regulation of progenitor cell markers in remodeled pulmonary arteries from patients with PAH, particularly in plexiform lesions, in addition to increased numbers of circulating angiogenic progenitor cells in patients with PAH (compared with control subjects). Functional studies of late outgrowth progenitor cells from patients with PAH that had concomitant BMPRII mutations revealed a hyperproliferative phenotype with impaired ability to form vascular networks. This study, together with recent experimental studies (40), adds important information to our current understanding of the remodeling processes in pulmonary vascular disease. Clinical trials, such as the Pulmonary Hypertension And Cell Therapy (PHACeT) Trial (ClinicalTrials.gov Identifier: NCT00469027), which is currently underway in Canada, will evaluate the effects of eNOS-transfected autologous endothelial progenitor cells delivered into the pulmonary circulation of patients with PAH. These studies may provide evidence for the therapeutic potential of cell-based therapies to modulate pathological vascular remodeling.

Tyrosine Kinases and Other Pro-proliferative Pathways

PAH is characterized by excessive vasoconstriction and thickening of vessel walls due to proliferation of vascular smooth muscle cells (SMCs). Several growth factors, including PDGF,

fibroblast growth factor 2 (41), and vascular endothelial growth factor, and more recently the noncanonical Wnt pathway (42), have been implicated in the abnormal proliferation and migration of pulmonary vascular cells. These growth factors act as potent mitogens and chemoattractants for SMCs, fibroblasts, and endothelial cells, usually forming homo- or heterodimers that stimulate cell surface receptors, which in turn activate the major signal transduction pathways, such as the Ras-mitogen activated protein kinase pathway. The cellular response to stimulation by growth factors involves proliferation, migration, and resistance to apoptosis. Several case reports suggested that in end-stage pulmonary hypertensive patients, compassionate treatment with the PDGF inhibitor imatinib could restore and/or improve clinical conditions (43–45). The phase III randomized controlled trial Imatinib in Pulmonary Arterial Hypertension (IMPRES, ClinicalTrials.gov Identifier: NCT00902174) is currently underway.

Serotonin (5-HT) and RhoA are mediators investigated now for many years in the context of vascular proliferation in pulmonary hypertension. Guilluy and colleagues in this journal described an intriguing cross-link between both signaling pathways (46). In lungs, platelets, and pulmonary artery SMCs from patients with idiopathic PAH and in mice with overexpression of the 5-HT transporter they found marked elevation in RhoA and Rho kinase activities and increases in 5-HT binding to RhoA. Moreover, the 5-HTT inhibitor fluoxetine and the type 2 transglutaminase inhibitor monodansylcadaverine prevented 5-HT-induced RhoA serotonylation and RhoA/Rho kinase activation, as well as 5-HT-induced proliferation of pulmonary artery smooth muscle cells. Treatment of mice with overexpression of the 5-HT transporter with inhibitors of the 5-HT transporter limited PH progression and RhoA/Rho kinase activation. The authors thus confirm that the 5-HT transporter/RhoA/Rho kinase signaling pathway is involved in 5-HT-mediated pulmonary smooth muscle proliferation and could be a promising target for new treatments for PAH.

Heme Oxygenase-2 and Large-Conductance Ca^{2+} -activated K^+ Channels: Lung Vascular Effects of Hypoxia

The oxygen-sensing mechanisms underlying hypoxia-induced pulmonary hypertension have not been fully elucidated. At the sensor level different candidates like mitochondria and NAD(P)H oxidases have been evaluated (47, 48). It was recently demonstrated that heme oxygenase 2 (HO-2) and BK channels ($\text{BK}\alpha$) can directly interact and form an oxygen-sensitive complex, suggested to play an important role in carotid body oxygen sensing. In addition, a role of HO-2 in the regulation of hypoxic pulmonary vasoconstriction was suggested. Against this background Roth and colleagues addressed the question of whether a complex of HO-2 and the BK channel may be important as an oxygen-sensing system underlying hypoxia-induced pulmonary hypertension and pulmonary vascular oxygen sensing (49). For this purpose investigations were performed using genetically modified mice in which HO-2 or the BK channel was deleted. They found that neither a lack of HO-2 nor of BK channels affected hypoxic pulmonary vasoconstriction or chronic hypoxia-induced pulmonary hypertension. Moreover, immunofluorescence staining and coimmunoprecipitation suggested only a weak interaction between HO-2 and $\text{BK}\alpha$ in pulmonary arterial SMCs. Thus they suggest that neither HO-2 nor $\text{BK}\alpha$ are involved in the pulmonary vascular oxygen sensing and signal transduction process underlying hypoxic pulmonary vasoconstriction or the development of hypoxia-induced pulmonary hypertension.

CONCLUSIONS

We have attempted to briefly highlight updates on PAH, from the *American Journal of Respiratory and Critical Care Medicine* and the broader literature, for 2009. Our review covers a broad swath of an energetic and expansive field, including recent changes in the classification of PAH, updates on major clinical trials of combination therapy, some of the important advances in diagnostic studies, and a review of novel basic science developments on mechanism and therapeutics. Our summary is necessarily limited and we encourage the reader to obtain the primary manuscripts and related work to examine in more detail.

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