Inaugural John Vane Memorial Symposium on Prostacyclin Science & Pulmonary Vascular Disease

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Proceedings

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WILLIAM HARVEY CONFERENCES

Contents

Foreword

John Vane and I had many shared interests including science, jazz and above all a vision of continuing achievement and exploration. It was through this shared vision that we first met, when I founded United Therapeutics and asked John to chair our Scientific Advisory Board, primarily due to his discovery of prostacyclin and its analogues including Remodulin.

John's discoveries, however, were not limited to the field of science; he had a great ability to find and nurture the talents of outstanding people. Over the years, he introduced me to many such individuals who have had a great influence on the work undertaken by United Therapeutics. This was one of many reasons why UT decided to provide an Educational Grant to fund a series of annual John-Vane-Memorial Symposia. The overwhelming response when people were invited to speak was one of enthusiasm and delight at being able to honour John's memory in such a positive manner. I am pleased to say that the symposium lived up to everyone's expectations and was a great success. This highlighter not only gives an insight into the proceedings of the two day meeting, but also clearly reflects our ongoing fight against Pulmonary Arterial Hypertension and the commitment and tenacity of researchers and physicians alike within the field. I would like to thank all the speakers who gave up their very precious time to participate. I know that John would have been delighted with the event. I very much look forward to the 2007 symposium and to working with William Harvey Research Ltd to take forward John's ethos of discovery.

Martine Rothblatt, PhD, JD, MBA
Chairman and Chief Executive Officer
United Therapeutics

John and Martine were friends who respected each other, both personally and professionally. They valued each others judgement, especially in the field of scientific research. The areas of this came from different aspects of the same subject which led to important developments in medicine. Curiosity is the spur which carries the researcher forward into "unknown lands". On occasion perhaps the happy accident occurs – serendipity.

This Inaugural Symposium in memory of John's birthday, was made possible by the thoughtful generosity of Martine. Lives are short, but from "remembered work" can spring fresh beginnings. So it was on the 29th March '06. Thank you Martine.

Daphne Vane
Lady Vane

Insights into Pulmonary Vascular Disease from Vascular Biology and Vascular Medicine

John P. Cooke MD PhD
Stanford University Medical Center

The life work of Sir John Vane was recognised in 1982 with the Nobel Prize for his discoveries concerning prostaglandins and related substances. His work on vasoactive substances in the pulmonary circulation also foreshadowed current clinical therapies for Pulmonary Arterial Hypertension (PAH). Exemplary of his prescience was the paper "Pressor effects of circulating endothelin are limited by its removal in the pulmonary circulation and by the release of prostacyclin and endothelium-derived relaxing factor" (*PNAS* 1988). Published almost 20 years ago, this work anticipated modern therapy using vasodilator prostanoids, endothelin antagonists, and nitric oxide (NO) therapy.

With respect to the latter, there is good support for an impairment of the synthesis and/or release of endogenous NO in PAH. Endothelium dependent vasodilation is impaired, and exhaled NO is reduced, in patients with PAH. The mechanism for this impairment of the NO synthase pathway is likely multifactorial. However, an emerging factor of interest is the endogenous NO synthase inhibitor ADMA (asymmetric dimethylarginine; for review, see Cooke JP, *Circulation* 2004). ADMA is an arginine analogue, that interferes with the y+ transporter (reducing arginine entry into the cell), and competitively blocks NO synthesis. ADMA is derived from the hydrolysis of proteins containing methylated arginine residues.

Plasma levels of ADMA are elevated in children with congenital shunts and PAH. In patients with idiopathic PAH, plasma ADMA levels are also increased; associated with unfavourable hemodynamics; and portend a poor prognosis. Plasma ADMA levels may accumulate with impaired function or reduced expression of the enzyme responsible for ADMA degradation, dimethylarginine dimethylaminohydrolase (DDAH). Emerging evidence from pre-clinical models of PAH indicate that a deficiency or impairment of DDAH is likely responsible for the elevated plasma ADMA levels observed in animals and man.

Parenthetically, we have developed a transgenic mouse model ubiquitously overexpressing DDAH. These mice have increased DDAH activity, and reduced plasma ADMA levels. Intriguingly, this effect was associated with increased plasma and urinary nitrogen oxides, and a modest reduction in systemic resistance and blood pressure (Dayoub H *et al*. *Circulation* 2003). These mice are protected from coronary vascular lesions in a murine model of transplant arteriopathy (Tanaka M *et al*. *Circulation* 2005). It is not known if these animals are resistant to pulmonary hypertension. However, unpublished data from the Vallance group suggests that a DDAH deficient mouse, with increased ADMA levels, may be susceptible to PAH. These pre-clinical studies provide new insights into the role of ADMA in regulating vascular tone and structure, and may be relevant to human pulmonary arterial hypertension.

Paediatric Manifestations of Pulmonary Vascular Disease and Early Intervention Strategies

Dr Robyn Barst
Columbia University College of Physicians and Surgeons

Pulmonary arterial hypertension (PAH), i.e. pulmonary vascular obstructive disease, is a debilitating progressive disease affecting patients of all ages, including children. Although paediatric PAH can be considered a distinct disease state, it shares a variety of features with adult PAH. Indeed, despite differences in clinical presentation, the pathology is similar in both children and adults with PAH. Paediatric and adult PAH also share a common clinical definition and method of diagnostic evaluation. On a structural level, the vascular lesions, including intimal and adventitial proliferation, smooth muscle hypertrophy, and *in situ* thrombosis, are seen in both patient populations. In addition, the same endothelial-derived vasoactive mediators, including nitric oxide, PDE_5, prostacyclin and endothelin, have been implicated in the pathophysiology of PAH in both children and adults. The current consensus suggests that PAH develops in individuals with a genetic predisposition, such as mutations in the BMPR2 gene, to respond adversely to a range of mechanical or chemical stimuli. When triggered, these predisposing factors initiate a cascade of deleterious events, often choreographed by endothelial-derived vasoactive mediators that lead to the development of pulmonary vascular obstructive disease. Moreover, therapies targeted to these mediators appear to be efficacious in PAH patients of all ages. These commonalities suggest a parallel underlying disease process in paediatric and adult PAH.

However, despite the similarities between children and adults with various forms of PAH, significant differences have been identified, with possibly more differences to be elucidated in the future. There is agreement on a less predictable course in children with PAH as compared to adults; untreated, children with Idiopathic Pulmonary Arterial Hypertension (IPAH) have a significantly higher mortality than adults with IPAH. However, with treatment, children appear to have a better prognosis than adults. Acute pulmonary vasoreactivity is higher in children than in adults, i.e. 30–40% at diagnosis vs. 7–10% in adults; however, the definition used to determine acute reactivity and treatment success in children is significantly different than that with adults as well as the parameters predicting long-term outcomes. And thus although currently the therapeutic algorithm for children with PAH is similar to that used in adults, we must remain cognisant to explore differences as opposed to focusing on similarities if we hope to improve our understanding of PAH in patients of all ages. In medicine, as in art, critical observation and exploration of the unusual is often far more elucidating that what first meets the eye.

Pulmonary Hypertension in Children

Prof Sheila G. Haworth
University of London

Pulmonary hypertension is more common in children than adults and has a more varied aetiology. Children suffer from Idiopathic Pulmonary Arterial Hypertension (IPAH), Familial and Sporadic, and Pulmonary Hypertension Associated (APH) most often with:

i) congenital heart disease, uncorrected and post-operative
ii) respiratory disorders including chronic lung disease, interstitial lung disease and congenital pulmonary hypoplasia
iii) connective tissue disease and the vasculitides.

Children with IPAH can have the same genetic abnormalities as adults with IPAH. In the UK we have established a Clinical Network to care for all children with pulmonary hypertension. Between April 2001 and June 2005 we treated 49 patients with IPAH, mean age at presentation 7.99 yr (range 0.1–19.7). 18 were male and 31 female. All were in WHO Functional Class III or IV with a pulmonary arterial pressure equal or greater than systemic arterial pressure. Mean pulmonary vascular resistance (PVR) was 21.65 units.m^2 (range 2.5–49). 46 children were treated with specific therapies; epoprostenol, bosentan and/or sildenafil in the presence of a fixed PVR (unresponsive to NO at cardiac catheterisation) and 4 positive responders were given nifedipine. The Kaplan-Meier survival estimates for our population were 84% at 1 yr and 76% at 3 yr. 5 children received a bilateral lung or heart/lung transplantation and are alive and well. A quality of life (QOL) survey for children (SF10) showed a median score of 27.15 (range 0–48.5) for physical ability and a median psychosocial score of 45.3 (range 19.6–60.7). A score $>$ 50 is considered normal.

We also treated 124 patients with APH. Survival was 89% at 1 yr and 79% at 3 yr. Specific therapies were given to 72%. The median QOL for the postoperative group was 33.8 (range 4.5–53.2) for physical ability and 43.6 (range 19.6–57.3) for psychosocial assessment. Median QOL scores for the Eisenmenger patients were 20 (range 0–45) physical and 34.5 (range 16.1–57.3) psychosocial. For both IPAH and APH, QOL scores did not relate to PVR, age at presentation or length of time treated.

Thus medical treatment improved survival in children with IPAH and in all cases of pulmonary hypertension psychosocial well-being improved in the face of physical restriction. In children with syncope and/or severe right heart failure can be helped by an atrial septostomy. We carried out 28 procedures with no mortality. Syncope was abolished and time to transplantation increased. Combination therapy +/- atrial septostomy produced the best therapeutic result.

Patients eventually fail to respond to epoprostenol. Fortunately however, we (L Clapp, E Falcetti and SGH) have recently shown that the smooth muscle cells of the explanted pulmonary hypertensive lungs of children who have been treated with epoprostenol for many years still express the prostacyclin receptor and that UT-15 will increase cAMP in these cells.

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Gene Therapy Strategies for ILD and IPH

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Pulmonary hypertension is a devastating disease which is characterised by critical insufficiency of the lung microcirculation. A novel approach for the treatment of this disorder is to address the problem of lack of effective microcirculation using a regenerative strategy. Our lab began to address this challenge using a gene therapy approach initially by transfecting somatic cells (i.e. fibroblasts or smooth muscle cells) with potential therapeutic genes. Our early experience confirmed that administration of smooth muscle cells transfected with endothelial nitric oxide synthase (eNOS) significantly attenuated the development of pulmonary hypertension in the rat monocrotaline model. This was associated with marked improvement in the perfusion of the lung microcirculation as demonstrated by a novel fluorescent microangiographic technique. By delaying this cell-based gene therapy to three weeks after administration of monocrotaline, we are also able to show that eNOS gene transfer significantly reversed established pulmonary arterial hypertension. Experiments were also performed to compare the potential efficacy of a variety of different genes that could have potentially beneficial effects on vascular remodelling and regeneration. Interestingly, cell-based gene transfer of the eNOS was consistently superior to other therapies including vascular endothelial growth factor (VEGF) both in reversing established pulmonary hypertension and in improving perfusion to the lung microcirculation.

More recently, we have focused on the use of endothelial progenitor cells (EPCs) which are believed to arise in the bone marrow and home to regions of vascular injury. EPCs participate in the repair and regeneration of systemic vasculature by both direct and paracrine mechanisms. We showed that EPCs by themselves were effective at preventing pulmonary arterial hypertension in response to monocrotaline. However, only EPCs transfected with eNOS were effective in significantly reversing established pulmonary hypertension, even restoring pulmonary hemodynamics to levels not different from control. This effect was also associated with dramatic improvement in perfusion and in a profound improvement in survival in this model.

Based on these encouraging pre-clinical data, a clinical trial is planned to assess the effect of eNOS transfected EPCs in patients with refractory idiopathic pulmonary arterial hypertension. The “Pulmonary Hypertension: Assessment of Cell Therapy (PHACET) Trial” is expected to start in the next month and will be the world’s first trial assessing regenerative cell therapy for pulmonary vascular disease and also the first trial examining the combination of gene and cell therapy for any cardiovascular disorder.

The Protective Role of Bone Morphogenetic Proteins in Pulmonary Arteries

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Mutations in the gene encoding for bone morphogenetic protein receptor-2 (BMPR-II) have been identified to be the inherited molecular mechanism for familial pulmonary arterial hypertension (FPAH). Although critical insights into the pathogenesis of pulmonary arterial hypertension (PAH) have been derived from reverse genetic studies investigating the familial form of PAH, the mechanisms through which BMPR-II receptor mutations cause the disease remain unclear. Little is known about the function of this receptor in the pulmonary circulation and the role of this mutation in vascular tone and structural remodelling in PAH/disease pathogenesis is unknown.

Endothelin-1 (ET-1) is a potent vasoconstrictor and comitogen for vascular smooth muscle, which has been implicated in the pathogenesis of PAH. BMP2, BMP4 and BMP7 were examined for their effects on ET-1 release by cultured pulmonary artery smooth muscle cells. In the presence of a concentration range of 100 ng/ml BMP7 for 24hr, ET-1 release was significantly reduced when induced by co-incubation with TNF-α and IFN-γ (both at 10 ng/ml). BMP2 and BMP4, had no effect on ET-1 release from these cells. The influence of BMPRII ligands on pulmonary vascular tone has not been investigated. Ring segments of PA set-up in organ baths, were assessed for their response to ET-1 in the presence and absence of 1, 10 or 100 ng/ml of BMP2, BMP4 and BMP7. Endothelin-1 (10^{-10}–10^{-7} M) gave a concentration dependent contraction in isolated PA. BMP7 inhibited contraction to ET-1 in a concentration dependent manner. BMP2 and BMP4 had no significant effect on contraction.

The ability of BMP7 to reverse TGF-β induced endothelial to mesenchymal transition (EMT) in pulmonary artery endothelial cells (PAECs). Using immunocytochemistry and western blotting, EMT was associated with a decrease in VE-cadherin expression, an endothelial cell adhesion molecule, inhibition of zonula occludens (ZO-1) protein expression and a loss of endothelial cell morphology. Incubation with BMP7 (100 ng/ml) reversed TGFβ-induced EMT, in association with re-expression of VE-cadherin, ZO-1 and restoration of endothelial phenotype. In addition, this study also demonstrates BMPRII ligands are able to produce these effects via Smad protein signaling in PASMC and PAECs.

These results suggest that BMP7 may have a role in regulating ET-1 release and ET-1 induced contraction in the pulmonary circulation. BMP7 is also able to maintain the morphology of pulmonary artery endothelial cells. Genetic mutations in BMPRII may lead to a loss of these regulatory mechanisms and contribute to the pathogenesis and vascular remodelling associated with FPAH.

Role of Erythrocytes in the Pathophysiology and Treatment of PPH

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Erythrocytes, by virtue of their ability to release ATP in response to physiological stimuli, participate in the local control of vascular calibre. In isolated rabbit lungs, erythrocytes of either rabbits or healthy humans are required to demonstrate flow-induced NO synthesis. ATP released from erythrocytes can activate endothelial purinergic receptors resulting in NO synthesis. A signal transduction pathway that relates physiological stimuli to ATP release has been described (Figure 1). Importantly, both Gs and phosphodiesterase 3B (PDE3B) are components of this pathway.

Previously, we reported that erythrocytes of humans with primary pulmonary hypertension (PPH) fail to release ATP in response to deformation possibly leading to a decreased stimulus for NO synthesis. In PPH, intravenous prostacyclin (PGI_2) is an effective therapy. PGI_2, after binding to its receptor, activates Gs resulting in increases in cAMP. PGI_2 is rapidly bound by erythrocytes. Here we investigated the hypotheses that the PGI_2 analogue, iloprost, stimulates cAMP accumulation in and ATP release from rabbit erythrocytes and that an inhibitor of PDE3B, cilostazol, potentiates this response (Figure 1).

Incubation of rabbit erythrocytes with iloprost (1–10 μM) resulted in increases in cAMP and ATP release. Importantly, iloprost (100 nM) produced vasodilation in isolated rabbit lungs perfused with washed rabbit erythrocytes in which resistance was increased by serotonin. However, in the presence of erythrocytes pretreated with glybenclamide to inhibit ATP release, the response to iloprost was prevented. These results are consistent with the hypothesis that, in the lung, a significant component of the vasodilator response to infused iloprost is mediated via erythrocyte-derived ATP. Thus, in PPH, infusion of PGI_2 may aid in correcting the defect in ATP release from erythrocytes associated with that condition. Finally, we incubated rabbit erythrocytes with cilostazol (CILO), an inhibitor of PDE3 used in the treatment of claudication. CILO (10 μM) increased basal cAMP levels as well as the increases in cAMP produced by iloprost. These studies suggest that the combination of PGI_2 and CILO might be of benefit to patients with PPH via an additive effect on ATP release from erythrocytes and could permit a reduction in the amount of PGI_2 that must be administered.

These results suggest that the erythrocyte, via its ability to release ATP, may be an important contributor to the control of vascular resistance in the lung. Moreover, by targeting the signal transduction pathway responsible for ATP release from these cells, new therapeutic approaches to the treatment of PPH may emerge.

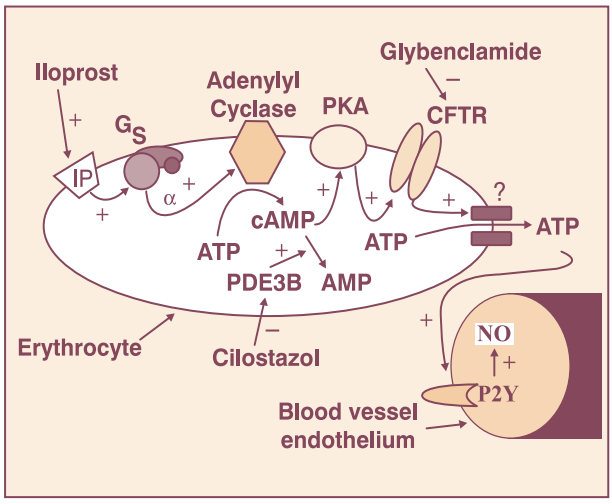

Figure 1:
IP = PGI_2 receptor; Gs = heterotrimeric G protein; PKA = protein kinase A; CFTR = cystic fibrosis transmembrane conductance regulator; PDE3B = phosphodiesterase 3B; P2Y = purinergic receptor

+ = stimulation; - = inhibition; ? = unknown ATP conduit.

Pathophysiology of Pulmonary Arterial Hypertension

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It is remarkable that extensive vascular remodelling in pulmonary arterial hypertension (PAH)[1] has actually little effect on pulmonary gas exchange. Studies using the sophisticated multiple inert gas elimination technique have shown that the distribution of ventilation/perfusion (VA/Q) relationships in these patients is close to normal,[2,3] the low-normal arterial PO_2 being essentially to be accounted for by a low mixed venous PO_2, consequence of a low cardiac output.[2,3] In some patients there is hypoxemia because of a right-to-left shunt through a patent foramen ovale.[3] In spite of vascular obliteration, physiological dead space remains normal, at rest as well as at exercise.[4] Therefore, the hyperventilation typically observed in PAH patients appear essentially determined by the altered function of the afterloaded right ventricle.

We recently reported that sympathetic nervous system activity, as assessed by peroneal nerve microneurography (MSNA), is markedly increased in patients with PAH.[5] Sympathetic overactivity is an important component of a neurohumoral activation in heart failure.[6–12] Sympathetic overactivation aggravates heart failure by increased metabolic demand of the failing myocardium, decreased myocardial oxygen supply, increased sodium and water retention, decreased skeletal muscle strength, increased ventilatory equivalents, decreased arrhythmogenic threshold, and myocardial as well as systemic vascular remodelling.[6–12] Accordingly, in heart failure, sympathetic overactivity is associated with a poor prognosis, and β-adrenergic blocking agents improve clinical state and survival.[12] In addition to the same deleterious effects as in recorded in congestive heart failure, sympathetic overactivity in PAH might directly aggravate the disease because of a contribution to pulmonary vascular remodelling.[13] However, β-adrenergic blocking agents are contraindicated in PAH patients because of the critical dependency of the afterloaded right ventricle on an adapted increase in contractile function.[14]

The mechanisms of sympathetic overactivity in heart failure are complex, and are believed to involve a decrease in cardiac output, atrial and ventricular wall stress, pulmonary arterial distension, decreased baroreflex sensitivity, increased muscle metaboreflex sensitivity, and positive interactions with the endothelin and renin-angiotensin-aldosterone systems.[6–11] Part of these derangements can be corrected by an atrial septostomy in patients with advanced PAH and refractory right ventricular failure.[15] The procedure has indeed been reported to improve clinical state, exercise capacity and survival, at the price however of a decrease in arterial oxygenation.[15] We have preliminary data showing that atrial septostomy decreases MSNA in PAH patients, and that this is closely correlated to associated decrease in right atrial pressure. This data suggests that at least part of increased sympathetic nervous system activity in PAH is related to right atrial distension. If confirmed, this would be the first report of a clinical relevance of the "Bainbridge reflex".[16]

PAH is a pulmonary disease with cardiac symptomatology.

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Deficiency of the VIP Gene as a Potential Cause of Idiopathic Pulmonary Hypertension

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Vasoactive Intestinal Peptide (VIP), a 28 amino-acid-residue peptide that belongs to the same family of peptides as secretin, glucagon, and Pituitary Adenylate Cyclase-Activating Peptide (PACAP), acts mainly by activating the adenylate cyclase-cyclic AMP system. Several observations suggest that VIP may play a critical role in the pathophysiology of Idiopathic Pulmonary Arterial Hypertension (IPAH).

VIP and the pulmonary circulation: Among the *actions* of VIP in the pulmonary circulation are: potent relaxation of pulmonary vascular tone, modulation of pulmonary vasoconstriction caused by hypoxia or by pharmacologic agents including endothelin, and inhibition of pulmonary vascular smooth muscle proliferation. Its *functional roles* include: co-transmission of pulmonary vascular relaxation, and modulation of pulmonary vasoconstriction and pulmonary vascular smooth muscle proliferation.

Features of IPAH in VIP knockout mice: We recently observed that mice with targeted deletion of the VIP gene show major features of PAH, including:

1) Pulmonary arterial & right ventricular (RV) hypertension (systolic pressure = 28.8 ± 0.7 vs. 18.7 ± 1.7 mm Hg in wild-type controls);
2) RV dilatation (assessed by echocardiography); and
3) Thickened, remodelled smaller pulmonary arteries, in the absence of arterial hypoxemia.

VIP is absent in patients with IPAH: VIP-containing nerves were recently reported absent in the pulmonary arteries of patients with IPAH, whereas such nerves are normally plentiful in those vessels.

VIP deficiency as a factor in the pathogenesis of IPAH: The expression of the IPAH phenotype in VIP knockout mice, and the absence of VIP nerves in pulmonary arteries of IPAH patients suggest that deficiency of the VIP gene may be causally related to the pathogenesis of IPAH. Validation of such a relationship would provide one more missing link in our knowledge of the genetic basis of the disease (Figure 1).

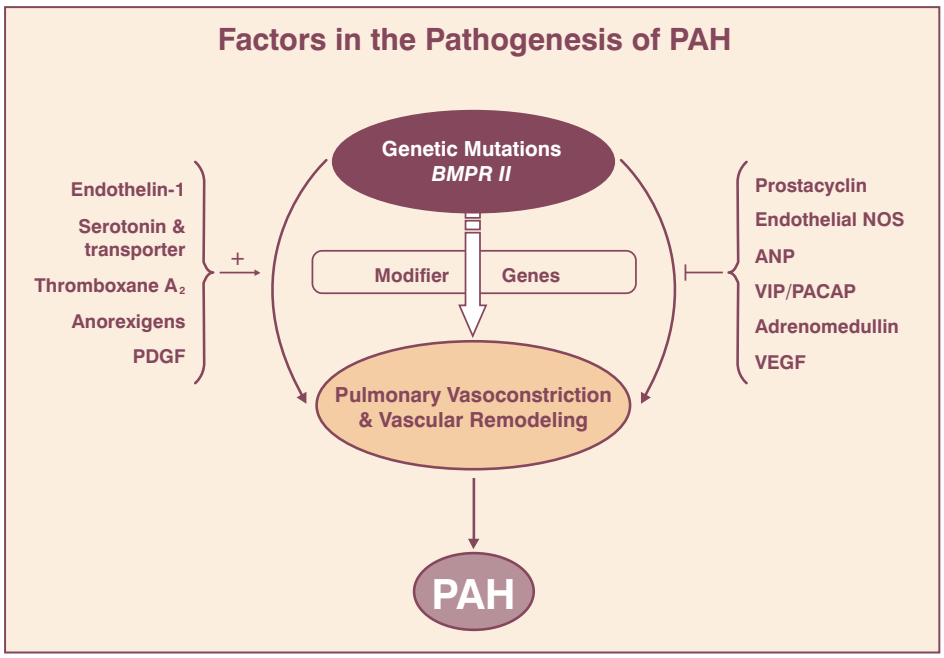

Figure 1: VIP as a drug for IPAH
Whether the lack of VIP is a cause of IPAH or merely an associated finding, the peptide promises to be an effective new therapeutic agent for the disease. Such a promise is based on its pulmonary vasodilator and antiproliferative effects, along with its anti-inflammatory and anti-apoptotic activities. A clinical trial of VIP, by inhalation, in IPAH patients has already yielded successful results.

Delegates at The Royal Society, London, UK

Prof Al Fishman (co-chair; left), Prof Sir Magdi Yaqoub (co-chair) and Prof Sheila G. Haworth

Cheryl Measures (conference organiser; left), Prof Chris Thiemermann (CEO, William Harvey Research Limited) and Lady Vane

Teresa Bongartz (United Therapeutics) and Prof Lewis Rubin

Prof Sir Magdi Yaqoub and Lady Vane

Nikki Vane (Sir John Vane's daughter) and Roger Jeffs PhD (President and Chief Operating Officer, United Therapeutics)

Implantable Gas Exchange Module as a Bridge to Lung Transplantation: Experimental Set-Up and Numerical Simulations with an Intravenous Gas Transfer Device

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The acute respiratory distress syndrome (ARDS) has been recognised as a cause of respiratory failure in patients with a variety of illnesses. Although we have learned much about the pathophysiology of this inflammatory syndrome since its earliest description, ARDS continues to claim the lives of 40–70% of its victims. Many treatment strategies have been used to prevent or treat ARDS.

Intravascular oxygenation and carbon dioxide removal remains a potentially attractive means of respiratory support in patients with acute or chronic respiratory failure. The first intravascular gas exchange device was originally conceived by Mortensen. This device is a temporary implantable device which is designed for use as a pulmonary assist device.

The objective of this dissertation is to characterise the oxygen supply and carbon dioxide removal capacity of a new intravascular gas exchange prototypes developed in our laboratory. Especially, attention is paid in the experimental approach and computational methods.

Considering the gas transfer limits of our first prototypes, computational tools are used to better understand gas exchange process between hollow fibres and surrounding blood. The application of computational modelling for gas transfer devices is relatively recent. Moreover, because of its complexity, simplified models are required. These numerical modes are suitable to highlight local gas transfer effects.

To conclude, modelling gas transfer between such a hollow fibre bundle and circulation blood remains a challenge for numerical community.

Clinical Experience in Combining Prostacyclin and its Analogs, Endothelin Receptor Antagonists, and Phosphodiesterase-5 Inhibitors in Pulmonary Artery Hypertension

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The development of drugs targeting specific pathogenic pathways in Pulmonary Arterial Hypertension (PAH) has heralded a new era in the treatment of this life-threatening disease. Current therapies address disease-specific abnormalities in the activities of three endothelium-derived mediators of vascular growth and reactivity: endothelin, prostacyclin, and nitric oxide. Clinical trials demonstrating improvement with agents targeting each of these pathways have led to regulatory approval of these drugs, yet many patients experience incomplete responses to monotherapy. Emerging experience suggests that strategies employing combinations of drugs, designed to target multiple pathogenic pathways, may result in greater improvement than with monotherapy. For example, small, uncontrolled trials combining prostanoids with endothelin receptor antagonists or Phosphodiesterase-5 inhibitors have shown potentiation of the clinical effects, including improved hemodynamics and exercise capacity. More formal randomised, double-blind, placebo-controlled trials combining multiple targeted therapies are underway. Unresolved questions, hopefully to be clarified by these trials, include:

1) Which pathways are the most important to address in PAH? Clinical experience suggests that prostacyclin and its analogues (epoprostenol, treprostinil, and iloprost, respectively) are among the most potent treatments for PAH and may, therefore, assume a central role in treatment strategies, particularly if these drugs can be successfully developed for administration by less invasive routes;
2) What is the preferred timing of initiation of combination therapy? The seriousness of PAH justifies early, aggressive approaches to treatment, yet the costs and complexity of these drugs argue for a layered approach, i.e. addition of second or third tier therapies only after monotherapy has failed to achieve a satisfactory response. Properly designed, long-term clinical trials are needed to address this question;
3) What end-points define a satisfactory clinical response? The short-term improvements in markers of disease severity that have been used as end-points for clinical trials, such as 6-minute walk, may be inadequate as sole indices of clinical response in individual patients. Achieving target improvements in multiple parameters that have been shown to be predictive of survival in PAH, including exercise capacity (6-minute walk $>$ 375m), Functional Class (NYHA Class II or better), and hemodynamic parameters (normalisation of cardiac output and right atrial pressure), may better define success or failure of a treatment strategy and guide future treatment.

Combinational Therapy Round Table Discussion

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There has been a great deal of advancement in the management of pulmonary hypertension in the last eight years. This is due to the introduction of prostacyclin therapy in its various forms (intravenous, inhaled and subcutaneous), and oral therapy as in various endothelial receptors antagonists and phosphodiesterase inhibitors. Most of these drugs were assessed in the double blind placebo controlled clinical trials and has shown a significant improvement in the mean six minute walk distances. However sub analysis of these trials and consequent clinical studies showed a great deal of variation in response and that a good percentage of patients with the clinical diagnosis of pulmonary arterial hypertension do not respond. It is becoming clear that pulmonary arterial hypertension is a very diverse disease and the response varies according to aetiology and the drug in use. There are no direct studies to characterise these responses and Figure 1 represents an approximate picture that need further study and confirmation.

The main issue is the need for combinational therapy; and if it will enhance the clinical outcome compared to monotherapy. One argument is that adding another mechanism to the already existing one will enhance the clinical outcome, as pulmonary hypertension is a multifactoral disease. Although this is attractive theoretically; there is no data to support it. Furthermore we do not have any proper clinical studies to show that combinational therapy can be more efficacious than monotherapy in any set of specific patients group. Some uncontrolled published data followed an "add-on" regimen and claimed reasonable enhancement in the clinical outcome, but failed to answer if there was a possibility that certain groups of patients will be better on one drug than the other and that adding the second drug may not be necessary. Once again we do not have controlled clinical studies to suggest that this is the case, although we can find anecdotal reports that replacing one drug and withdrawing the failed medication can be done safety.

So the questions for the roundtable can be summarised in the following:

- Why do we need "add on" to the failed treatment?
- Can we stop the failed medications?
- Do we have evidence the failed medications cause any remodelling? and stopping will reverse that remodelling?
- Do all patients of various pathologies respond to the same class of drugs?
- If not why do we put our effort in large clinical trials that only concentrate on "add-on" rather than *differential effect according to the aetiologies?*
- Do clinical trials of "add-on" have any clinical morbidity or mortality benefits?

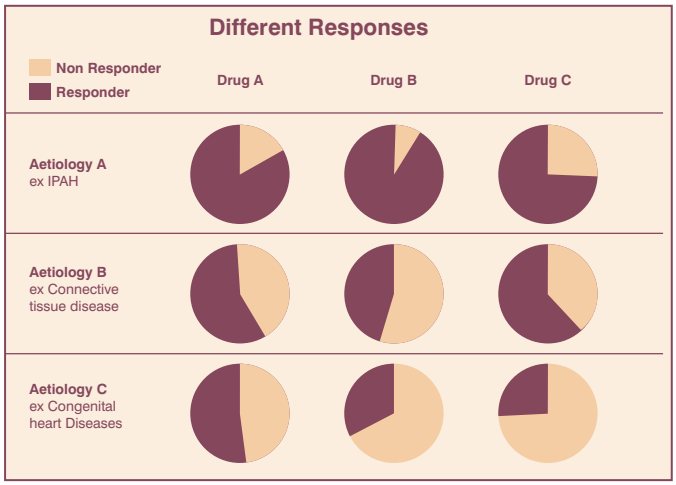

Figure 1

Genetic Determinants of Penetrance in PAH

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Several potential causes of variable phenotype and reduced penetrance exist for autosomal dominant traits. These include alternative alleles (allelic heterogeneity), alternative genetic loci, modifier genes and environmental factors. An example of a genetic modifier in humans is the decreased amount of sickle hemoglobin that is seen in patients with sickle trait who also are heterozygous for alpha thalassemia trait. Genetic modifiers can affect not only penetrance but also severity of expression.

Studies of the estrogen receptors, signaling and estrogen response elements in the BMPR2 promoter are needed to determine if they contribute to the female preponderance seen in all forms of PAH. Somatic mutations in the normal BMPR2 genes of those heterozygous for one BMPR2 mutation in lung tissue could cause loss of heterozygosity, although the evidence is against this. While mutational differences in the promoter and other regions of BMPR2, and in potential modifier genes may be found, the functional importance of these variations will have to be demonstrated.

PAH is a complex genetic disease. Except for gender, there are no known influences on penetrance. In non-familial PAH, clearcut environmental and biological triggers exist, including appetite suppressants, HIV infection, portal hypertension and collagen vascular disease. The best documented environmental associations are fenfluramine and stimulants such as amphetamines. Studies are needed to look at parity, cigarette smoke, depression, hormone therapy, childhood infections, hypertension, activity, altitude dwelling, diet, medicines, lifestyles and other potential influences on development of disease.

The search for modifying genes is just underway. One approach is to pick candidate genes that have a role in vascular structure and function and also possess functional polymorphisms that are relatively frequent in the population. These include the serotonin transporter, nitric oxide synthases, vasoactive intestinal polypeptide, the urea cycle enzyme carbamyl phosphate synthetase, endothelin receptors and ion channels, among many. HIV has a well documented association with PPH, and HHV8 may be a cofactor in the lungs. Figure 1 shows the major conditions in which PAH can occur and suggests some modifying genes, and other biological and external stimuli that could result in disease. Studies of the effects of genotype on response to therapy are needed.

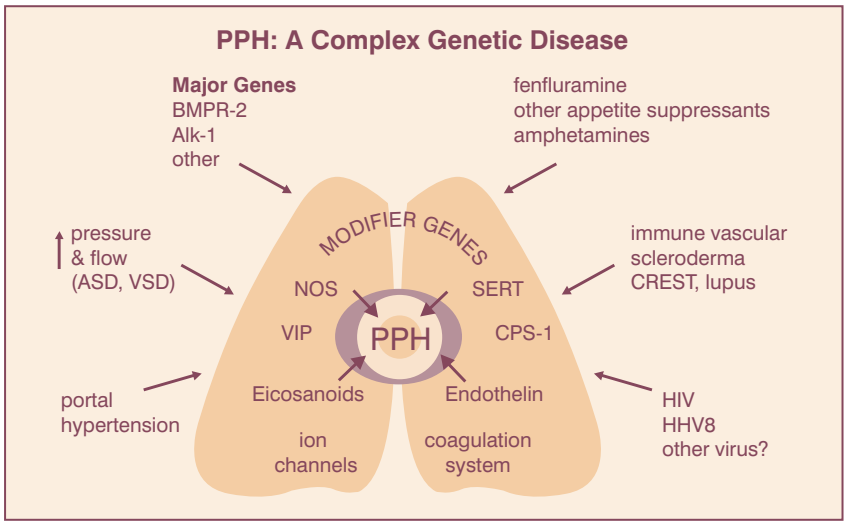

Figure 1

Scleroderma Associated Pulmonary Arterial Hypertension

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One of the most lethal complications of systemic sclerosis is pulmonary hypertension. This can occur in the context of established interstitial lung fibrosis (secondary pulmonary hypertension) or without evidence of significant interstitial fibrosis – termed isolated pulmonary hypertension. Overall prevalence of pulmonary hypertension is estimated to be around 15% of cases of SSc. Diagnosis at present requires right heart catheterisation. Non-invasive tests are being developed and an algorithm based upon Doppler-echocardiography, pulmonary function indices and possibly serum levels of N-terminal pro-BNP (N-pro-BNP) is likely to be used in screening.

Management of SSc associated pulmonary hypertension has been stimulated by developments of therapies that are able to influence outcome in idiopathic pulmonary hypertension. Exercise capacity is generally assessed using the distance walked in six minutes under standard conditions (SMWT). This has prognostic implications and is used for risk stratification. Oral anticoagulation, spironolactone and oxygen supplementation are used as supportive background therapy in pulmonary hypertension in SSc. Although therapies are available for SSc associated PAH, analogous to idiopathic PAH in general they are given only for advanced disease. Earlier intervention may be advantageous and is currently under investigation. Options for Class III disease include oral bosentan and sildenafil. Alternative include inhaled or subcutaneous prostacylin analogues. Intravenous agents are generally reserved to progressive disease due to the substantial additional problems associated with this including catastrophic rebound pulmonary hypertension upon withdrawal and line-associated sepsis. Failure to respond or progression on single agent therapy usually prompts addition of a second drug. Combination treatment often continues although it may be possible to slowly reduce or cease the first agent.

Management of SSc-PAH associated with pulmonary fibrosis is especially challenging. Prognosis is especially poor in this group of patients but clinically meaningful improvement can occur after treatment with therapies developed for PAH. Although outcome is especially poor in the subgroup of SSc with both PAH and PF such cases can gain significant exercise capacity. It is likely that some patients have a vasculopathy which is independent of the interstitial fibrosis and this can respond to therapy.

Overall there is now a better short-term outcome in SSc associated pulmonary hypertension and survival is improved relative to historical controls. Moreover, changes in levels of N-pro-BNP associate with survival – falls in serum level predicting good outcome and rising levels predicting poor survival. At present most of the survival benefit occurs in advanced isolated PAH in SSc.

In the future it is likely that early detection and treatment will be beneficial and that new therapies will be used sequentially and in combination. Controlled trials to define long term benefits of combination therapy in SSc-PAH and in pulmonary hypertension in SSc associated lung fibrosis are needed.

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Treatment of Pulmonary Hypertension due to Interstitial Lung Disease

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The quality of life and survival of patients with WHO Group 1 pulmonary arterial hypertension (PAH) has improved significantly, thanks to the availability of many new, effective, and safe PAH-specific therapies. However, many other patients suffer from pulmonary hypertension (PH), including WHO Group 3 PH patients with hypoxaemia due to underlying lung disease, such as chronic obstructive pulmonary disease and interstitial lung disease (ILD).

The prevalence and severity of PH in patients with lung disease depends upon the severity of the underlying lung disease and the related gas-exchange disturbances, as well as the population studied. For example, the prevalence of PH in ILD patients ranges from 30–100%, being lower in patients with early or mild ILD, and virtually 100% in ILD patients being considered for lung transplantation. Importantly, the pathophysiology of PH in patients with lung disease may be multifactorial, as other causes of PH may contribute, including left ventricular disease (WHO Group 2 pulmonary venous hypertension) and pulmonary thromboembolic disease (WHO Group 4 PH).

Therapeutic choices for patients with lung disease and PH are currently limited. There have been very few trials of the novel PAH-specific therapies in this patient population. Case series, uncontrolled observations, and small cohort studies suggest beneficial effects of therapies such as prostaglandin analogues (e.g. intravenous epoprostenol, inhaled iloprost), phosphodiesterase-V inhibitors (e.g. Sildenafil), and endothelin-receptor antagonists (e.g. bosentan) in ILD patients with PH. However, data are limited and methodologically weak. Moreover, worsening oxygenation due to pulmonary vasodilation and ventilation: perfusion mismatching has been described.

In order to better define the use of novel PAH therapies in WHO Group 3 PH patients, including those with ILD-associated PH, many questions need to be addressed by well-designed randomised, placebo-controlled clinical trials. These include:

1) Which biologic pathways and targeted therapeutic agents are of greatest scientific relevance;
2) Which clinical endpoints will best assess the response to PH-therapies in patients with symptoms and limitations due to underlying lung disease;
3) Which is the preferred route of administration of PAH therapy, based on consideration of optimal clinical efficacy and the least adverse effects;
4) What is the potential for exacerbation of underlying lung disease;
5) Are there clinically-relevant pharmacokinetic and pharmacodynamic interactions of PH-specific therapies with other respiratory therapies?

The success of novel therapies for PAH patients has led to cautious optimism for future treatment of other groups of patients with PH, including those with underlying lung disease.

Prospects of Prostacyclin or its Analogs in Treating Subtypes of Left Heart Failure

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Congestive heart failure, either systolic or diastolic, is a common cause of pulmonary venous hypertension. Chronic elevation of left heart filling pressures from either heart failure or mitral or aortic valvular disease in most instances results in "passive pulmonary hypertension", a condition in which pulmonary artery pressures are elevated yet the transpulmonary gradient is normal. A subgroup of patients with chronically elevated left heart filling pressure develop more severe pulmonary hypertension, with an elevated transpulmonary gradient and microscopic changes in the pulmonary vasculature which are quite similar to those seen in idiopathic pulmonary arterial hypertension, sometimes referred to as "reactive pulmonary hypertension". Why this subgroup is predisposed to these changes is unclear. The development of significant pulmonary hypertension in the setting of left heart failure negatively impacts the prognosis.

Treatment of left ventricular systolic dysfunction has been well described and medical therapies include diuretics, digitalis, ACE inhibitors, beta-blockers, and in some cases, inotropic infusions. More advanced therapies such as mechanical assist devices and cardiac transplantation are indicated in some circumstances. Treatment of left ventricular diastolic dysfunction has been less well described, but principles include optimal control of systemic blood pressure and other risk factors, tight regulation of volume status, maintenance of sinus rhythm, and pharmacologic therapies such as ACE inhibitors which may favourably alter ventricular remodelling. Pulmonary venous hypertension that results from aortic or mitral valve disease may be treated by correction of the underlying valvular lesion in most instances.

The acute hemodynamic effects of prostacyclins, specifically epoprostenol, have been reported in four studies of patients with congestive heart failure. In the series, epoprostenol resulted in a 30–60% increase in cardiac index, a 17–36% reduction in pulmonary capillary wedge pressure, a 5–7% increase in heart rate, and a 10–23% reduction in systemic blood pressure. The largest randomised chronic trial in patients with systolic dysfunction was the FIRST trial, in which 471 patients with an ejection fraction of $< 30\%$ were enrolled. While there was no difference in the six minute walk distance endpoint, a higher mortality was observed in the patients treated with epoprostenol.

While it is unlikely that prostacyclins will be studied again in the congestive heart failure population in general, an argument might be made to study prostacyclins in those with pulmonary hypertension "out of proportion" to the elevation of left heart filling pressures. Such a study will be complicated, and patients should only be considered candidates if they have significant pulmonary hypertension after the left heart disease is optimally treated.

Sustained Release Formulation of Prostacyclin Analogs – Is Long-Term Zero-Order Release on the Horizon?

Roger Jeffs PhD.
on behalf of United Therapeutics Corporation

The benefit of prostacyclin and its analogues is now well-established in the treatment of patients with Pulmonary Arterial Hypertension (PAH). Currently, prostacyclin therapies are approved for delivery via intravenous, subcutaneous and inhalation routes. The development of an effective and well-tolerated orally bioavailable form of prostacyclin, however, has proven elusive. In particular, issues related to poor oral bioavailability, lack of sustained release, short half-life of the active moiety, and high peak plasma concentrations have limited therapeutic utility due to insufficient efficacy and/or poor tolerability.

Treprostinil sodium is the bioactive ingredient of Remodulin, an approved therapy for subcutaneous and intravenous use in patients with PAH. UT-15C is the diethanolamine salt of treprostinil. UT-15C has been formulated as a solid oral dosage form using an osmotic tablet technology. The goal of this technology is to provide an oral dosage form with pH-independent sustained release over a preset interval (approximately 8–10 hours) sufficient for twice-daily dosing.

As shown in Figure 1, preliminary Phase 1 results in normal volunteers given a single dose of the osmotic tablet (dark and light burgundy curves) demonstrate a diminished Cmax compared to an immediate-release formulation given four times (yellow triangles), a prolonged half-life (approximately 9–11 hours), and a prolonged period with a constant rate of release. A positive food interaction is present as co-administration with food further prolongs the length of exposure.

Based on the favourable kinetic profile of this SR oral dosage form of treprostinil, clinical trials in patients with PAH are being initiated.

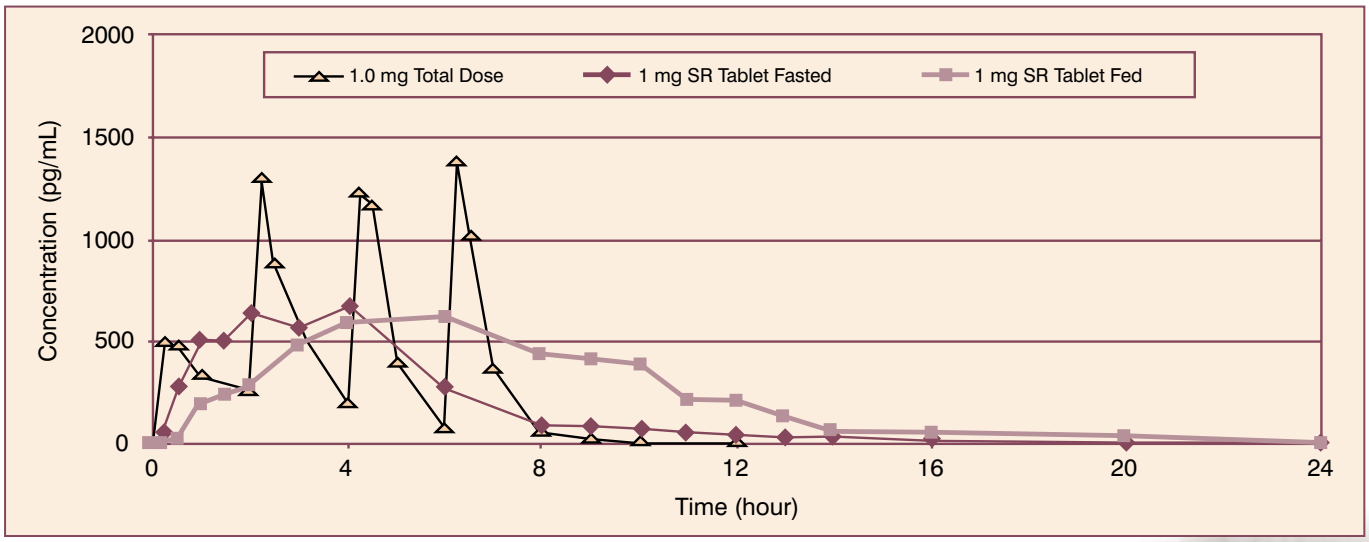
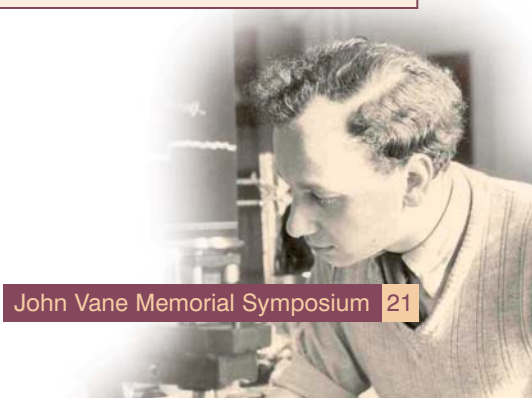


Figure 1



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