Today's work, is tomorrow's possibility.
The Journal

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# Table of contents

## 1. Editorial

Evolving PVRI: One step closer to enhancing our understanding of pulmonary vascular disease  
Sachindra Raj Joshi  

## 2. PVRI News & Activities

8th PVRI Annual World Congress on Pulmonary Vascular Disease: A brief report  
Oleg Pak, Djuro Kosanovic  

Scientific Meeting Review China  
- Reflections on China and an inspirational session on “Jūshixibào” the “macrophage”  
  Eileen Bauer  

Sino-German workshop on high altitude adaptation  
- Akylbek Sydykov, Xuebin Qi, Lan Zhao, Martin Wilkins, Friedrich Grimminger, Hossein Ardeschir Ghofrani, Chaoying Cui  

The First Latin American Symposium on Pulmonary Hypertension in Children: A brief report  
Gabriel F. Diaz G  

Interview  
- Pulmonary Vascular Disease and the Birth of the PVRI:  
  Ghazwan Butrous and Michael Seimetz  

## 3. Journal Club

**Interactive Discussion:**

Is dietary L-carnitine a strategy to combat COPD-induced muscle wasting?  
- Robert Ringseis, Klaus Eder, Djuro Kosanovic, Ralph Theo Schermuly, Norbert Weissmann, Michael Seimetz  

Dysregulated inflammatory response in pulmonary hypertension is still enigmatic: what about psoriasin?  
- Djuro Kosanovic, Michael Seimetz, Oleg Pak, Himal Luitel, Akylbek Sydykov, Norbert Weissmann, Ralph Theo Schermuly  

## 4. Art Club

Infography  
- Abnormal right ventricular relaxation in pulmonary hypertension  
  Rebecca Vanderpool
5. Learners’ Corner

Perspective
The study of high altitude pulmonary arterial hypertension at the Chilean Andean Altiplano
Emilio A. Herrera, Germán Ebensperger,
Roberto V. Reyes, Aníbal J. Llanos

Did you know?
The fluid extravasation which leads to pulmonary edema may occur across large vessels as well as capillaries?
Kevin Lowe, MD,PhD.

Review
Advances in understanding of pulmonary arterial hypertension and the evolution of Experimental pulmonary hypertension models
Michiel Alexander de Raaf, Norbert F. Voelkel, Harm Jan Bogaard

6. Clinical Corner

Case Report
Chronic thromboembolic pulmonary hypertension in a young patient
Vaclavik, Lindsay MD and Safdar, Zeenat MD

7. PVRI General Updates

New PVRI Member of Staff - Aaron Shefras, PVRI Executive Administrator & Marketing Officer
Stephanie Barwick, Chief Executive Officer, PVRI
Evolving PVRI: One step closer to enhancing our understanding of Pulmonary Vascular Disease

by Sachindra Joshi

From its inception, PVRI is concerned with enhancing the understanding of the complex pulmonary vascular diseases (PVDs). There are three key primary objectives of the institution: to improve the care of patients all over the world, who suffer from PVDs, to facilitate and accelerate research in the clinical and basic science of the PVDs, and to encourage the development of new therapies for PVDs. Through constant improvement, PVRI has evolved to best achieve its primary objectives. The improved PVRI website and the new logo of the PVRI are the clear evidences of the phenotypic evolution of the PVRI. The changes in the core of the PVRI such as the new PVRI Constitution and organizational structure, the new Committee for Young Clinicians & Scientists, previously known as the “PVRI Young Council”, and establishment of new PVRI Task Forces are some of the aspects of genotypic evolution.

From the beginning of this year, through various meetings, discussions and interviews at the 8th PVRI Annual World Congress, PVRI has come one step closer in better understanding the pathogenesis of PVDs and significant improvements of the current therapies. Drs. Oleg Pak and Djuro Kosanovic have summarized the activities of the 8th PVRI Annual World Congress in the PVRI News and Activities section. Bringing together basic scientists and clinicians investigating the molecular mechanisms behind the pathobiology of pulmonary vascular disease is of utmost importance as it applies to better understanding of the disease and more importantly to find new and effective therapeutics for the PVDs. The establishment of a new PVRI Task Force, Preclinical and Molecular Science, led by Prof. Mandy MacLean- an eminent scholar in the field of PVDs - is an excellent example of PVRI stepping forward in enhancing the understanding of PVDs.

PVRI together with the Saudi Association for Pulmonary Hypertension (SAPH) at the 8th Annual Joint Pulmonary Hypertension Assembly further augmented our understanding of PVDs by elaborately discussing CTEPH, PH in the young, PAH in congenital heart diseases, management challenges in PAH patients and pregnancy challenges in PH patients as well as practical clinical scenarios and more specific PAH management updates & challenges and PH in the developing world: Diagnostic and Management Dilemmas. These discussions are available online as a part of our learning section on the PVRI website. To fight a complex disorder like PVDs, a collective effort in understanding the disease pathology and the potential therapeutic options is important. Therefore, PVRI has brought together the pharmaceutical industries, regulatory authorities, clinicians and basic scientists under one umbrella for the second time during the Annual Drug Discovery and Development Symposium in London.

PVRI has moved further towards advancing the understanding of PVD through its Central Asia Task Force, now referred to as “CAPH”. (Central Asia Pulmonary Hypertension) This is facilitated by Drs. Stefano Ghio from Italy, Majdy Idrees and Tarek Kashour from Saudi Arabia and Ghazwan Butrous from the UK, who held the first successful Master Class on PVD. PVRI successfully conducted theoretical and practical sessions on echocardiography and right heart catheterization. Dr. Stefano Ghio conducted an Echo/Doppler examination on patients with suspected pulmonary hypertension and right heart catheterization was conducted under the supervision and instruction of Dr. Tarek Kashour. Apart from educational and clinical activities, PVRI also held its first fundraising event, the PVRI Charity Concert, in Canterbury, United Kingdom on May 5th 2015, in honor of “World Pulmonary Hypertension Day”. Over 200 people attended the concert, and the PVRI was
PVRI Chronicle, as a brand ambassador of PVRI, is upholding the Institute’s mission statement by focusing on the traditional triad of research, education, and clinical care, in activity and report alike, presents you with news and activities from PVRI to interactive discussions and case reports in the field of PVDs. This issue includes an interview with Prof. Ghazwan Butrous, in which he summarizes pulmonary vascular disease in the early days and the birth of the PVRI, Prof. Ghazwan says that “...science is a collaborative work- so you obviously like to work with other centers and scientists and exchange ideas...” but he felt the lack of a forum for collaborative work in particular to pulmonary vascular disease and this void lead to the birth of the PVRI. For the Journal club section, we have selected two interactive discussions covering dysregulated inflammatory response in pulmonary hypertension and dietary L-carnitine: a strategy to combat COPD-induced muscle wasting. The Learners’ Corner presents a review article on advances in understanding of pulmonary arterial hypertension and the evolution of experimental pulmonary hypertension models; a perspective on the study of high altitude pulmonary arterial hypertension at the Chilean Andean Altiplano and a ‘Did You Know’ article on pulmonary edema. The Clinical Corner presents an interesting case report on chronic thromboembolic pulmonary hypertension in a young patient.

Taken together, with the new changes and the collective effort of PVRI fellows, we are clearly one step closer to enhancing our understanding of pulmonary vascular disease which comprises of: improving the care of pulmonary vascular disease patients, facilitating and accelerating research in the clinical and basic science of the disease, and encouraging the development of new therapies.
The 8th Annual World Congress On PVD

All our collaborators.

One Event.

Paul Corris, future PVRI President 2016/17
The 8th Annual World Congress on Pulmonary Vascular Disease

by Oleg Pak

The 8th PVRI Annual World Congress on Pulmonary Vascular Disease was held this year in the beautiful and futuristic city Guangzhou, China on January 15th - 18th 2015. The congress was hosted by the Dong Fang Hotel, and it was organized as a joint meeting together with the 7th National Chinese Congress on Pulmonary Embolism and Pulmonary Vascular Diseases, creating a unique milieu to meet and unite both the Western and Eastern civilizations. Such a multicultural concept resulted in great enthusiasm amongst attendees who took this opportunity to discuss the most important and urgent scientific and clinical issues in the field of pulmonary vascular disease (PVD), and to share their valuable experiences.

The conference sessions covered a variety of scientific and clinical topics, giving the particular attention to the most interesting and ‘hot’ achievements and novel findings in the last years with regard to PVD. For example, alteration of the MicroRNAs system was described in the PVD pathology and these molecular regulators received a considerable focus in the recent past, and the lectures from Drs. Schermuly, Chan and Chun provided further insights on their role and potential therapeutic strategies, considering both, the pulmonary vasculature and right heart remodeling/failure. Furthermore, abnormally activated immune system and inflammation were recognized as culprits in the development of pulmonary hypertension (PH).

Among different inflammatory cells, the macrophages were of particular interest during this congress, as evident from valuable talks of Drs. Zheng, Stenmark, Johns and Leopold. The group of the current clinical classification of PH also received a noticeable attention, and Drs. Weissmann and Aldashev shared their knowledge and expertise on the pulmonary vascular abnormalities in the context of COPD and high altitude environment, respectively.

Following the heritage and trace of Dr. Robyn Barst in pediatric research, and knowing that PH in children is very frequent across the world and there is no satisfactory therapy lectures from Drs. Adatia, del Cerro, Diaz and Ivy further enlightened the current issues on pediatric registries, clinical trials and potential therapeutic approaches. The distinctive ‘face’ of the PH pathology in women, including all aspects from pregnancy, sex differences and hormones, contraception etc., was the subject of the talks from Drs. Cockrill, MacLean, Shafer, Preston and Mandel.

The patients’ registries and novel opinions on clinical trials, covering a variety of indispensable experiences, perspectives and knowledge from all over the world (Africa, Asia, Europe, South America), took a large portion of the congress and resulted in interesting and intrigued talks/discussions given by Drs. Thienemann, Wen, Jian-Guo, Kerkar, Laliberte, Corris, Diaz and MacDonald. Finally, the non-pharmacologic
approaches to PH, re-synchronizing the right ventricle, and compliance, cardiac output, ventriculo-vascular coupling as targets of choice etc. were the subjects covered by the lectures from Drs. Boogard, Pritzker, Moledina and Thenappan.

Finally, our PVRI Annual General Meeting was also held during the congress. Briefly, the PVRI members discussed different aspects covering the topics from the branding and communication (updated PVRI logo, promotional materials, educational website, social media presence…) to fundraising (development of the committee, establishment of a future fundraising strategies and organization of more frequent fundraising events).

In conclusion, the 8th PVRI Annual World Congress on Pulmonary Vascular Disease has definitely achieved much more than expected, ultimately leading to the better understanding of PVD pathogenesis and significant improvements of the current therapies. However, we still have a long way to go in order to possess in our hands the satisfactory curative strategies and options.
PVRI News & Activities

Mandy MacLean, leader of our newest Task Force: Preclinical & Molecular Science, speaks at the conference.

PVRI Council Members

The three wise men: From left, Stelios Orfanos, Djuro Kosanovic and Ghazwan Butrous

One of Guanzhou’s prettiest sites; the famous TV Tower sits above the city.

One of Guanzhou’s prettiest sites; the famous TV Tower sits above the city.
The conference hall starts to fill up as the meeting commences on the second day.

Questions are taken on the floor and an engaging discussion takes place as a conference talk draws to a close.
Nick Morrell and Bert van den Bergh collect their welcome packs & name tags as the conference kicks off.

Each of the delegates prepare for their talk, and as one prepares for his photo.
Eileen Bauer, our Co-Editor in chief with some of the delegates from the Chinese PHA. In order to help spread awareness of the disease, the patient association encourages people to wear blue lipstick, representing lack of oxygen in patients. You can find out more about the Chinese PHA by emailing this address:

phachina@yahoo.com.cn
We arrived in mid January in Guangzhou, China for PVRI’s 8th Annual World Congress. Having already started our New Year in the Western world, our visit to Guangzhou occurred just prior to China’s turn into its new zodiac calendar year: the year of the sheep. And as customary, beautiful red envelopes embossed with golden signs were displayed and available for purchase everywhere - waiting in anticipation to be filled with money, to be given as gifts of good fortune to family and friends for the coming new year.

As I was reading through the meeting’s program, enjoying the warm weather of Southern China compared to the icy conditions of the Eastern US, I became excited about a session on macrophages. While the drug industry, in large, is still targeting the vasodilatory pathways for the treatment of patients with pulmonary hypertension, these same patients, sadly, despite current drugs, still succumb to disease. Targeting this route alone, in my opinion, is clearly not the path to a cure. Dr. Rubin Tuder already observed and reported decades ago on the presence of immune cells in the plexiform lesions of patients with pulmonary hypertension. His work, in part, led me to focus our research on the role of the immune system in pulmonary vascular disease.

I thus happily volunteered to write about this very interesting, highly informative, and inspiring session on “Macrophage diversity in inflammation-related pulmonary vascular remodeling”. The session included five presentations, with the following titles 1) The many faces of macrophage activation (Dr. Limin Zheng, Director for Tumor Biotherapy, Sun Yat-sen University, Guangzhou, China) 2) ‘Immune cell cross-talk with endothelium and vascular smooth muscle’ 3) ‘Macrophages and monocytes in experimental pulmonary hypertension’ 4) The macrophage in human pulmonary hypertension—a case of scleroderma and 5) Putting the brakes on macrophages in pulmonary hypertension (Dr. John Rogers, Professor in Anesthesiology and Critical Care Medicine at Johns Hopkins University, US). Since PVRI only received authorization to video-record from two of the presenters, I will cover only these two talks here: the keynote address presented by Dr. Zheng and the final presentation of the session by Dr. Rogers.

Macrophage “Basics” – With its roots in the Greek - macro meaning ‘large’ and phage meaning ‘to devour’ - the term at its simplest describes a large cell that is able to devour cellular content. Elie Metchnikoff, a Russian bacteriologist, was credited with the discovery of the macrophage in 1884, sharing the Nobel Price in Physiology or Medicine with Paul Ehrlich in 1908 “in recognition of their work on immunity”. Though originally described only based on its phagocytic function, the macrophage is now thought of as one of the most plastic cells in the hematopoietic system, and its complexity is still not fully understood.

The journey of the macrophage begins as a bone marrow derived cell called a monocyte. As it matures the monocyte enters the blood stream with the majority of monocytes taking up residence in specific sites, silently waiting at their final destination for an activation signal. In this fashion alveolar macrophages reside in the lung, while Kupffer cells are the resident macrophages of the liver, Langerhans cells in the skin, microglia cells in the nervous system, and osteoclasts in the bone. However, a much smaller group of monocytes remains in the blood, freely circulating, and waiting for chemotactic clues to guide them...
to the site of infection where they transform into a macrophage, invade the tissue and do their job. While the life span of a monocyte is rather short-lived (maximum of about 3 days), a transformed macrophage can live for months, even years.

Macrophages have been studied for over 100 years. A PubMed search using the keyword “macrophage” resulted in numerous reports demonstrating the importance and influence of this cell in biology and disease (see Fig 1).

Another quick online search revealed the existence of a journal carrying just the single-worded title: ‘Macrophage’ – a peer-reviewed open access journal. Scientists even created a community based website dedicated to macrophages (http://www.macrophages.com/) – a centralized resource connecting scientists worldwide in their common interest on macrophage biology.

While far from new to immunologists, I was happy to hear and learn how the macrophage is now also entering our world of pulmonary vascular disease, including one of my main interests, the proliferative disorder of pulmonary hypertension.

Keynote Presentation by Dr. Zheng

In his keynote address “The Many Faces of Macrophage Activation”, Dr. Zheng clearly demonstrated the plasticity and diversity of the macrophage using examples from his field of tumor immunology and cancer immunotherapy. He divided his talk into three parts: 1) an introduction to monocytes/macrophages, 2) a presentation of data on how macrophages contribute and promote disease and tumor progression, and 3) a presentation of data to support macrophages’ ability to change function based on location and additional environmental factors. Dr. Zheng pointed out that the potential of our increasing knowledge hopefully will allow us to redirect the “negative” macrophage function in one area of the tumor to its positive function in a separately distinct area within the same tumor.

Historically immunologists grouped macrophages phenotypically into 2 subsets: M1-like activated macrophages representing classical activation, and M2-like activated macrophage – activated by IL-4 and mainly responsible for T cell remodeling within tissue, like the heart and vasculature. Although well supported and understood in mice, this simplified view is not fully supported to hold true in humans. Markers like iNOS activation and arginase are important in the murine macrophage biology, but these markers seem to be absent in human macrophage biology. And, like all science, also macrophage biology evolves as new evidences are discovered.

Using atherosclerosis as an example, Dr. Zheng pointed out the macrophage’s well-established contributory role to disease. In atherosclerosis macrophages were believed to have no ability to proliferate. However, increasing recent evidence
suggests that indeed, these aortic macrophages do proliferate as shown by several groups using Ki67 staining and BrdU uptake. What pathways lead to macrophage activation? Based on the classical view, a macrophage is the result of a differentiated monocyte. On the other hand, there also exists the potential of macrophage self-renewal.

Dr. Zheng continued to demonstrate the diversity of macrophage function with examples from his field in tumor immunology. A tumor infiltrating macrophage is able to cause inflammation, producing IL12, to act immunosuppressive, activating arginase and producing IL10. It is able to promote angiogenesis and tumor growth, as well as invasiveness of the cells. Dr. Zheng presented data of positive macrophage staining throughout the entire tumor. However, positive staining for macrophage activation alone, was limited only to the peritumoral stroma, while the rest of the tumor contained immunosuppressive macrophages expressing IL4. How does the tumor educate the macrophage? In vitro experiments revealed a temporal component. The macrophage, once activated, changes over time into an immunosuppressive state including elevated IL-10 expression. This is comparable to Lipopolysaccharide (LPS) tolerance in humans, where we observe an early strong inflammatory response that switches over time.

The questions that then arise are: Is macrophage activation a host defense mechanism? And what role does it play in tumor progression? Dr. Zheng explained that a subset of T- cells, expressing IL-17, strongly promote angiogenesis with an observed positive correlation between CD68+ and IL-17+ cells in the peritumoral stroma, however, without any apparent association in the intratumoral region. To further elucidate this potential link, his team treated macrophages with tumor-supernatant, waited six days to allow them to change into the immunosuppressive phenotype, and then examined their ability to trigger a Th17 T- cell expansion. Repeating these studies with activated macrophages showed that activated cells, in comparison to immunosuppressive ones, are superior in causing this Th17 response and expressing elevated levels of interferon-Y. We begin to get a glimpse of the complexity of the macrophage and its changing roles based on its position and environment.

In addition, autocrine cytokines produced by the tumor itself, like TNFα and IL-10, transiently lead to increased expression of Programmed-death 1 ligand (PDL1) in macrophages and is highly enriched in the peritumoral stroma of patients. PDL1 is thought to protect tumor cells from being targeted by the immune system. To further complicate our understanding of macrophage biology, there can be large differences between a macrophage’s in vivo biology compared to its in vitro behavior. For example, indoleamine 2,3 dioxygenase expression (IDO) is strongly expressed in macrophages and monocytes in human tumor tissue in vivo but is undetectable in macrophages used for in vitro studies. Why? The macrophage reacts and interacts very dynamically to and with its environment. It fine-tunes when and where it needs to be immunosuppressive versus activated. This plasticity seems to be one major requirement to be a well functioning immune cell. The in vitro loss of IDO expression in macrophages, for example, is due to an absence of T-cells in cell culture in laboratory bench experiments. When viewed in a broader context, these findings, give us important insights. The knowledge hopefully allows us in the future to take advantage of the plasticity of the macrophage by shifting its state to the one that is desirable for us to help prevent and cure disease.

**Concluding Presentation by Dr. Rogers**

Dr. Rogers concluded the macrophage session with an excellent presentation entitled, “Putting the brakes on macrophages and immune response in pulmonary hypertension”. He highlighted
current progress on targeted immune therapy for the treatment of pulmonary hypertension. In agreement with my personal view, Dr. Rogers pointed out that targeting the Nitric Oxide (NO) pathway alone does not present a successful treatment or cure for patients with pulmonary hypertension – though still continuously exploited by industry. As Dr. Zheng had explained earlier, Dr. Rogers also pointed to the well-established role of macrophages in the vascular disease atherosclerosis. In atherosclerosis several subsets of macrophages have been identified, displaying clearly the continuous and plastic phenotype a macrophage can inhabit.

Macrophages are very responsive to external stimuli, such as chemokines, adhesion molecules, or other inflammatory mediators. Using these to our advantage might allow us to use macrophages as a new therapeutic target that hopefully will result in better treatment options for our patients. Dr. Rogers described several approaches currently used by institutions and companies, i.e. the creation of small molecule inhibitors, the targeting of transcription factors, and lastly the creation of antibodies. As of now ten antibodies are available for therapy in the US cumulating in over 400 clinical trials. The approach is relatively easy. In the cancer field companies have already progressed from a single target antibody to create bi-specific antibodies: for example targeting VEGF in addition to Interleukin-6. Dr. Rogers described various methods of antibody production, such as creating mouse monoclonal antibodies followed by humanization to allow for higher affinity. Another more recent approach is to begin the work using a humanized mouse. While there are over 590 pulmonary hypertension clinical trials targeting the vasodilatory prostacyclin pathway, only 21 trials have targets outside the traditional research field, like the immune system.

As Dr. Rogers pointed out, lungs from patients can display fibrosis in addition to elevated levels of a large range of chemo- and cytokines. Furthermore, fibrosis is a Th2 macrophage response. Since targeting an entire pathway is difficult and often leads to extensive side effects, targeting of specific cells would thus be a better option. As part of his work, he has previously identified that the protein hypoxia-induced mitogenic factor (HIMF) is upregulated by a Th2 stimulus as well as hypoxia and is furthermore strongly present in the pulmonary vasculature. HIMF belongs to the FIZZ/resistin/RELMbeta protein family, has adipokine and insulin resistance properties, and may thus mediate vascular pathology associated with obesity and metabolic syndrome. Dr. Rogers observed “massive release” of HIMF into cell media after hypoxic exposure as well as its upregulation in the vasculature itself. Additionally, HIMF/Resistin seems to be particularly relevant in the lung, only being upregulated under specific stresses, and could thus present a good target to minimize side effects. Dr. Rogers presented findings that blocking HIMF leads to attenuate disease in rodent models of pulmonary hypertension using measures of pulmonary remodeling, right ventricular systolic pressure, Fulton index, and cardiac output to evaluate severity of disease. Furthermore, injection of HIMF into mice will induce experimental pulmonary hypertension and is associated with an increase in Interleukin-6 (IL-6) production. Overexpression of IL-6 itself has already been reported to lead to experimental pulmonary hypertension in mouse models. On the other hand, the lack of HIMF in null mice leads to attenuated disease compared to wildtype controls.

Originally HIMF was described as chemokine mediating myeloid cell chemotaxis. In this pulmonary context HIMF has been shown to lead to IL- 4 dependent macrophage recruitment as injection of HIMF itself showed no effect in mice lacking IL- 4. Dr. Rogers’ team further observed that
Tail vein administration of HIMF into mice caused significant whole heart hypertrophy after 16 weeks. In more relevant cases of human scleroderma, heart biopsies revealed increased levels of Resistin-like molecule (RELM β), rising with heart failure but dropping with unloading. Dr. Roger’s team is currently exploring the role of RELM β in patients with pulmonary hypertension and he shared at this meeting his latest findings on RELM β’s increased expression in pulmonary plexiform lesions, in dendritic cells as well as in pulmonary vascular smooth muscle cells. Furthermore, serum resistin levels can be used to predict mortality in human PAH (IPAH and SSc PAH) and show correlation with the 6 min walk test. Based on all of these supporting data, Dr. Rogers was excited to share with the group that he is currently in the process of creating therapeutic antibodies and he hopes to push his project as fast as possible from the bench to bedside.

The Take-Home Lesson

This session on macrophages in pulmonary vascular disease was highly informative and the science presented by all the participants was clearly on the cutting edge of how to approach pulmonary disease. What did we learn?

1) The potential for a single immune cell, the macrophage, to play a significant role in pulmonary vascular disease, a disease not traditionally thought of as being immune-mediated. 2) The macrophage, and most likely all immune cells, possess tremendous plasticity. 3) As our understanding of this plasticity increases it might allow is to use the macrophage as therapeutic tool, so that we can artificially shift its phenotype and use its natural function at the time and location we want to.

I know that my fellow colleagues in the PVRI Committee for Young Clinicians and Scientists were as excited as I was about the session on macrophages in vascular disease, and I hope that this excitement will carry over into our research and give us a closer look of the role immune system in pulmonary hypertension.

Thank you all for organizing this meeting in China: XieXie – Thank you.

Eileen Bauer, PhD
Department of Surgery School of Medicine University of Pittsburgh; Vascular Medicine Institute, University of Pittsburgh, US
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Fellowship Opportunity

Ian Adatia has kindly highlighted a 2 year fellowship opportunity for all young researchers. The fellowship runs for 2 years starting in July 2016. All applications can be forwarded to:

iadatia@ualberta.ca

More info:
http://pvri.info/content/fellowship-opportunity-stollery-childrens-hospital
The PVRI 9th Annual World Congress on Pulmonary Vascular Disease 2016

Start the year with us.

The PVRI would like to invite you to our 9th Annual World Congress, which will take place in Rome, Italy in January 2016.

Registration will open soon, so check back on our website for the scientific program, hotel registration and abstract submissions.

Scientific Agenda Including:

- Beginnings: genes, foetuses, metabolism.
- Guidelines & PH in LV dysfunction.
- Imaging: routine/molecular /3-D reconstruction/pathology.
- Combination therapy and the Potts shunt.
- Targeting PVD; metabolism, antibodies, metals & ...
- Do rats and mice remotely resemble us? Translational relevance.
- Moderated posters + Meet the experts.

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Registration fees

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It is estimated that over 140 million people live above 2500m in various regions of the world. There are many challenges to living at high altitude, but chronic hypoxic exposure is prominent among them. How well healthy humans adapt to hypoxia depends upon the severity of exposure and their genetic background. Pulmonary hypertension (PH) is a complication of life at high altitude. Its severity varies between individuals and there is evidence for genetic adaptation. PH together with increased erythropoiesis, place an increased pressure load on the right ventricle. This reduces exercise capacity and can lead to heart failure and premature death. Understanding the factors that enable adaptation to hypoxia could provide insight into strategies and novel therapies that might not only facilitate adaptation and improve health in high altitude residents but also improve the management of hypoxic illness at lower altitudes.

From November 24th to 26th 2014, the first Sino-German workshop on “Pathomechanisms of High Altitude Pulmonary Hypertension and Genetic Basis of Adaption to High Altitude Hypoxia” was held at the Excellence Cluster Cardio-Pulmonary Systems (ECCPS), University of Giessen, Germany. This workshop was funded by the Sino-German Center for Research Promotion, and co-organized by Professor Hossein Ardeschir Ghofrani of University of Giessen, Germany and Professor Chaoying Cui of Tibet University, China. The main objective of this meeting was to bring together German and Chinese scientists with a common interest and complementary expertise in pulmonary vascular biology and high altitude life to develop a joint collaborative programme to better understand the genetic and environmental
Leshen Chen introduces the Sino-German Center for Research Promotion and presents the funding schemes of the German Research Foundation.

Prof. Cui gives an overview on high altitude research in Tibet.

Rory Morty introduces the MBML (Molecular Biology and Medicine of the Lung) International Graduate Programme at the University of Giessen.
factors contributing to high altitude PH.

Before the workshop started, Prof. Werner Seeger and Prof. Hossein Ardeschir Ghofrani of University of Giessen, gave an introduction of the aims and objectives of this meeting and provided an overview of the main research activities at the ECCPS. Prof. Leshen Chen introduced the Sino-German Center for Research Promotion and explained the funding schemes of the German Research Foundation. During the workshop, experts and scholars from universities and research institutions in Germany and China, with some prominent international invited speakers from Switzerland, Chile and Belgium, presented their findings and had scientific discussions that addressed major research and clinical topics in the field of PH and high altitude medicine and biology.

The workshop covered a vast range of topics, including genetics of adaptation to high altitude, pathophysiology and molecular mechanisms of pulmonary hypertension in humans and animals, epidemiology and clinical aspects of high altitude diseases. The workshop provided an excellent platform to connect top researchers from German and Chinese universities and research institutions, with some prominent international invited speakers and fostered several scientific collaborations between the leading scientists from Germany and China. The workshop has achieved its goal of forming research partnerships and identifying specific research topics in the area of PH and high altitude medicine and biology. The workshop has been a gratifying opportunity to strengthen the interaction between China and Germany in this research area that has not only local and national significance, but is also of great global importance. We envision this partnership to develop into a long-term collaborative research initiative for the next three to five years.

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Audience during scientific sessions.
Participants continue discussions in a relaxed atmosphere during coffee breaks.

From left to right: Norbert Weissmann, Emilio Herrera and Akylbek Sydykov discuss potential collaboration between University of Giessen and Universidad de Chile.

From left to right:
Himal Luitel, Rory Morty, Djuro Kosanovic and Ralph Schemuly discuss future collaboration between University of Giessen and Rampur University in Chitwan, Nepal.

President of Giessen University, Joybrato Mukherjee (fifth from left) attended the bilateral discussion between Giessen University and Tibet University after the Symposium.
Taking into account the increasing interest in Latin America for Pulmonary Hypertension (PH), largely due to the Continuing Educational Programs in different Latin American countries on pulmonary hypertension in general and specifically in children in Colombia, we saw it convenient to organize the First Latin American Symposium on Pulmonary Hypertension in Children in Colombia. This idea had the immediate approval and support of the PVRI and the Colombian Society of Cardiology whose President Dr. Efrain Gomez decided to give all the administration support for the its organization. Then, the dates and place of this event, were defined: Hotel Hilton in Cartagena de Indias on February 21st - 22nd, 2014.

For the development of the project, the great collaboration of Prof. Ghazwan Butrous and Prof. Sheila Glennis Haworth (current President of the PVRI) was very important. They, together with Prof. Ian Adatia (Alberta University, Edmonton Canada), Prof. Antonio Augusto Lopes, Head of Pediatric Cardiology, Heart Institute (INCOR), Sao Paulo Brazil, Dr. Maurice Beghetti, Head of
Pediatric Cardiology, Heart Institute (INCOR), Sao Paulo Brazil, Dr. Maurice Beghetti, Head of Pediatric Cardiology, University of Geneve, Geneve Switzerland, Dr. Maria Jesus del Cerro, Head of Pediatric Cardiology, Centro Especial Ramón y Cajal, Madrid España and Dr. Julio Sandoval Head of Research, Instituto Nacional de Cardiologia de México, México DF, immediately accepted to collaborate as speakers. With Prof. Haworth's collaboration, the draft of the program was made and everyone of the speakers contributed to the final program that covered the different topics related to PH in children, from the newborn (Persistent Pulmonary Hypertension of the Newborn), to the adult with PH related congenital heart disease. In the program, a lecture by Migdalia Dennis, President of the Latin Society of PH for patients, was included.

The Symposium was projected with two objectives: 1) To increase the interest on PH in children in Latin America and 2) to promote the research in this interesting topic, having as central issue, the importance of early detection of PH to avoid the pulmonary vascular disease. This was a very important aspect, as was reflected in the Logo of the Symposium, in which you can see the sentry box of the Cartagena walls that represent the pulmonary trunk which give origin to the pulmonary branches with pulmonary

Mona Butrous, Ghazwan Butrous, Sheila Glennis Haworth and Ian Adatia
vascular disease, complication that we need to avoid. To promote the research, we gave great relevance to the Poster Session that was included in the scientific program. As an incentive, we received the money to give as a prize to the five best posters. The organization of the event required hard work for one and a half years.

The Symposium began with a simple opening ceremony on February 20th at 7:30pm in the Hilton Hotel near the beach. There were short talks from Dr. Gabriel Diaz, President of the Symposium, Dr. Efrain Gomez, President of the Colombian Society of Cardiology and Prof. Sheila Glennis Haworth, President of the PVRI. After that, there was a cocktail reception and a show of typical Caribbean dances.

The scientific program began on Friday 21st 2014 at 8:00 AM and ran until 6:00 PM with active participation of 147 participants (mainly Pediatric Cardiologists and Pediatric Pulmonologists) from different countries: Argentina, Brazil, Chile, Peru, Ecuador, Venezuela, Honduras, Mexico, USA and a nourished Colombian participation. At noon and for two hours, the Poster Session was developed with a satisfying participation: 34 posters from different countries. The reviewers were the invited speakers and they selected the top five.

At 7 PM there was an elegant dinner supported by one of the economic sponsors, in the Chapel of the Santa Clara Hotel (former Convent of the Poor Clares) in the beautiful Colonial Cartagena de Indias (inside the walled city).

On Saturday 22nd, the scientific program began at 7:00 AM with a work breakfast and despite the early hour, there was active participation of around 110 attendants. The central topic discussed was how to organize networks and registries on PH in Latin America, based on the experience of Dr. Maria Jesus Del Cerro in Spain, who was the speaker. This will be the basis for the development of the registry of PH in Children in Latin America. Then the scientific program continued, ending at 2:00 PM. It is important to note the permanent attendance of the participants; the conference room was full all the time.

We consider that the First Latin American Symposium on Pulmonary Hypertension in Children was a complete success. The two objectives set at the beginning of the project were fulfilled. All the participants and the speakers were completely satisfied.

This First Symposium on Pulmonary Hypertension in Children will be the basis for continuing the work on PH in Children in Latin America in the future, trying to achieve its two objectives: To increase the interest on Pulmonary Hypertension in Children and to promote the research in this interesting topic. We propose to organize this Symposium every two years and the next location being Buenos Aires, Argentina. Dr. Dora Fabiana Haag was nominated as President of the Symposium.
The Second Symposium on Pulmonary Hypertension will be in March or April 2017. The final date will be confirmed in the near future.

**Gabriel F. Diaz**

Department of Pediatrics
Universidad Nacional De Colombia
President of the First Latin American Symposium on Pulmonary Hypertension in Children
Cartagena de Indias

*Gabriel Diaz introduces a speaker at the event*
PVRI News & Activities

Pulmonary Vascular Disease and the birth of the PVRI: An interview with Prof. Ghazwan Butrous by Dr. Michael Seimetz

MS: Professor Butrous, thank you for the opportunity to have this interview with you- it is truly an honor.

You were one of the founders of the PVRI; you have been the Managing Director, the Treasurer, and now you are the PVRI President Emeritus. What was your initial idea when you founded the PVRI? How did you recognize the need for the Institute?

GB: It is an interesting story, because at the time I was at Pfizer and working on pulmonary hypertension programs and developing Sildenafil- but that’s another story. I started to notice that pulmonary vascular disease was a new area. In the nineties nobody talked about pulmonary hypertension because there were no drugs. But now we had a few drugs. But the interest and the science did not have a real network in which to work together, because as you know, we have the American Cardiology Society, the European Society of Cardiology, but for pulmonary hypertension there was no such thing.

The other thing I noticed is that science is a collaborative work- so you obviously like to work with other centers and scientists and exchange ideas. But there was no such forum available. I had the idea of creating a small network- but I wasn't thinking about a big organization.

So one day, I still remember it, I was in a meeting for another function, and Ardi Ghofrani- Professor Ardeschir Ghofrani from Giessen- and Professor Martin Wilkins were both in the meeting. I took them aside and I said, ‘By the way, how about we start a small network between a few centers so that we can work and exchange ideas?’ And they said, “That’s a very good idea, let’s think about it.” Of course, I’m well-known to be a determined person, so I said “Well, let’s take it further.”

That was actually in 2005 around the fall. So the three of us began to talk, and decided to get more people together to discuss this small network. I began to think whom I could involve, and I thought about friends and colleagues. I called Mark Semigran, Evangelos Michelakis, Stuart Rich, of course Ardi Ghofrani and Friedrich Grimminger, and they all liked the idea. So we decided to meet, but of course we didn't have any money as no one was supporting us, so we all decided to pay for ourselves and meet at Heathrow airport. So indeed, we met at the Holiday Inn at Heathrow airport, and stayed there for two days. We started to develop the idea of this network. We didn't have a name for it at that time at all. We spent about two days talking and we decided that it would be good to have a network in which we could work together, and we wanted to register it as an educational program, and to see if we could call it an institute. Of course it's not easy to call something an institute, as you need approval from the UK government to be involved in educational activities. Martin (Wilkins) took that upon himself and later on, to make a long story short, he obtained the approval from the ministry to name our burgeoning network as an ‘Institute’.

But we needed to discuss it further, so we decided to meet whenever it was next possible. This turned out to be in February 2006 during the ATS meeting in New Orleans, and there Marlene Rabinovitch joined us as well at the local Holiday Inn. And that was the first time that we started to think as a proper institute, and think on the global issues rather than our personal research projects.
Of course we needed a name, and someone came up with 'PVRI' or Pulmonary Vascular Research Institute - and it fits the other PVRI, which is of course the Pulmonary Vascular Resistance Index. I registered it, and we decided it was time to get more people in. We invited 25 people to meet in Malta in 2007, and that is our first official meeting. We met in Malta for two full days, and I put forward the ideas on how the PVRI works - its Task Forces, the colleagues, no hierarchy, everybody working together. And everyone was so excited about it - at least, these 25 people!

We decided not to ask people to join directly, because we did not want to be like an invitation-only club. Instead, if people wanted to join, they would be welcome. Martin spoke to Professor Chen Wang from China who was very excited by the initiative, and he said that he would establish a PVRI China Centre. And then he invited us all to hold the first meeting in China, and this happened to be in Xian - and this was the first time we had 600 people attend a PVRI meeting! Much like this meeting here in Guangzhou!

The following year we decided to have the annual meeting - but this was not meant to be scientific. I mentioned to a colleague that it may be nice to have a scientific meeting also, to encourage people to attend. In effect, we had one whole day for the Annual General Meeting (AGM) and another day for scientific sessions; this was first established in Marbella. Then everybody got excited, and every year it grew, slowly, slowly. And now to my surprise, it has become too big! People started to love it! After Marbella we began having discussions during the meetings, so everybody felt - and feels - like they are contributing to the subject. From there it grew, and every year we had more and more people, and you know the story.

In 2010, we decided that we needed somebody to help us. I used to employ a couple of temporary administrators, until we found Nikki Krol, who became the first permanent one. And that's how it happened.

MS: That's a really amazing story. You mentioned that you started with only a few people, and now the number of members is increasing and increasing. You also mentioned some rather famous people. What do you think - why does the PVRI attract young scientists and experts in the field?

GB: I think the main secret - and without a doubt, this is what I truly believe - is that the PVRI is a flat organization - everybody is important. There is no hierarchy. We've always said that a PVRI fellow is any person who works - if you don't work, even if you are a Nobel Laureate, then you are not important to us. And of course, when you've got a lot of energetic young scientists, who are ambitious and they want to do some work - they will contribute. In fact, the young people have contributed in a very real way in building the PVRI. Many people tell me, 'when I attend the PVRI meeting, I feel like I am being listened to, rather than at other meetings where some speakers are up on a podium and everybody else is silent...'. I believe that the idea of that exchange creates a lot of potential, and people start to enjoy and enrich the whole thing. I think that the success of the PVRI is not in the so-called founders, but rather in the people who joined it and made it happen, and I hope it will continue in this way.

MS: I'm pretty sure it will, based on the success of previous meetings. Earlier you mentioned sildenafil- a drug that was initially used for erectile dysfunction, and which is now a standard therapy for pulmonary hypertension. Somehow, it
is relatively unknown that you in fact discovered that sildenafil can be used in lung diseases.

GB: Well, it’s one of the more interesting stories of my professional life. I am a cardiologist, and my training in cardiology focused on cardiac arrhythmias. Later in my career, I was asked to become a scientific advisor to Pfizer, mainly in the cardiac arrhythmia area because they were trying to develop drugs for this disease. At the time my job was mainly to give ideas and evaluate points.

You probably know how the process of drug approval works in a pharmaceutical company: the drug usually stays in clinical development, and the scientists will do Phase I followed by Phase II and then Phase III, and when it becomes a drug it will go to the marketing site. So when Viagra was approved for erectile dysfunction, one of the directors in Pfizer came to me and said, “you know Ghazwan, Viagra is no longer with us but there may be some other applications for it- so can you think about what those may be?” To be honest, I had no idea about pulmonary hypertension at that time. But as I like to joke, I went to my monastic cell and closed the door for about three months- literally three months- just looking at the literature about PDE5 and PDE5 inhibitors.

Eventually I created three or four ideas, and started to write them down for the director. I had a couple of chapters which all focused on different ideas for Viagra applications- and one looked at the potential use for Viagra in pulmonary hypertension. That was actually chapter three! So there were another two ideas that I can’t discuss here.

The reason I thought about the possibility of a link wasn’t due to some miraculous discovery- rather, it was like all proper scientific work- a meticulous process. I noticed that a drug called zaprinast, which was produced by May & Baker before it was discontinued, was a PDE5 inhibitor and was used to treat pulmonary hypertension in hypoxia. And then I noticed that PDE5 is overexpressed in the lungs, and to my own discovery as I didn’t know that previously, I found that PDE5 is present in the lungs more than in the penile system. By then there were a few papers on zaprinast, which was the precursor to sildenafil as the latter was developed from zaprinast. So I realized that there was good science behind the theory. People were talking about cyclic GMP which hadn’t happened before, but erectile dysfunction brought it to light and increased interest. So I wrote a chapter and I discussed it with the director. He appeared interested so I suggested we produce a project in the cath lab. Of course there was no budget for it, but he managed to get a little bit of money together, a few thousand dollars, and I contacted five or six investigators which I knew in England, and asked if they wanted to help us. I won’t mention the name, but one of the top investigators in pulmonary hypertension at that time said that our idea was interesting, and wanted to know the name of the drug. When I answered “Viagra”, he started to laugh and asked if I was joking!

This happened in the mid-nineties, and at the time we could only treat very severely ill patients with pulmonary hypertension as there were no drugs available. And this investigator referenced this, and said ‘these patients are dying in the cath lab, and if I give them Viagra, you know what will be all over the news the next day!’ I said ‘Listen, it works.’

I knew we had an excellent rationale behind it and
and there was no reason not to do it. The investigator didn’t say no, but it was clear he didn’t want to be involved. Nonetheless, he wanted to help me and was likely a little curious. So he gave it to a patient with COPD—and we now know that COPD patients are generally least responsive to the drug. But by chance this patient showed a 50% decrease in the pulmonary pressure. So he phoned me in the evening and said ‘Ghazwan you’re absolutely right! I’ve never seen a drug like that!’ and I said ‘I told you the science would work.’

In 1998, he actually started to prescribe it to patients but he didn’t tell me as it was an off-label thing. I only discovered this was happening six months later when one of the patients was travelling and needed a bigger supply. I have the results of all these patients—they all improved their six minute walk test.

By then I had about 12 patients and they all showed good results. People were starting to get encouraged—but not everybody. After all, it’s Viagra—it’s considered a lifestyle drug. Again and again I heard that ‘You cannot give it for life threatening conditions.’ In 1999 people were starting to take note of the on-going research, and it was mentioned at the American Heart meeting, a small meeting for pulmonary hypertension attended by about 50 people. In response, the audience started laughing—loudly—because we were giving Viagra.

But to answer your question: I was the person who was on the receiving end of all the information, which allowed me to develop this idea. One of the things I always say to young scientists is to be persistent. If I hadn’t been persistent about this, none of this would have happened; everything would be dead. Even many people in Pfizer weren’t sure about this project, because the drug is for erectile dysfunction and not for this kind of thing, and it hadn’t been done before which meant many investigators did not believe in it. But some case reports started appearing here and there. The first case report was a small little letter that appeared in the New England Journal of Medicine in 2000. The patient was almost dying and was even rejected for heart transplantation. So the doctor at that time gave him 500 mg of Viagra. And the patient started to improve! This encouraged the doctor to write the case report. I didn’t know about that, and just read about in the New England Journal like everybody else. So I phoned the doctor and I said, “in my experience, that dosage is too high.” And he said “I don’t know about dosage, so I just gave it like that.”

So I said, “from what we’ve seen in the cath labs, 75 mg is enough.”

By the way, the patient is still alive and here now, even though he was on the transplant list at the time way back in 2000.

So these kinds of reports, popping up here and there, encouraged other doctors to try giving Viagra for pulmonary hypertension. And I started receiving thank you letters from patients, whom gave me a lot of impetus. This was crucial to me, because two years after we began investigating it, there was the possibility that the program would be axed and we would no longer offer the medication. I started to fight and said “what about all these patients— you cannot just deny these things.” I still remember, it was an agony for me to continue.

But anyway, out of this came a miracle. Eventually
the company was convinced and, I have to say it publically because I’m proud that he did it, Dr. Declan Doogan became the president of this research. He decided that it was worthy of becoming a program, and created it himself. This led to Phase III, and within two years, despite many investigators believing that it would never include patients, the drug was approved. And following this approval, it became the number one drug for pulmonary hypertension even until now.

Admittedly, it’s the most commonly used drug mainly because it was the cheapest drug at that time.

MS: And because it works.

GB: Yes, and because it was available all over the world because of erectile dysfunction. It’s not the only nor the most ideal drug- but for that time it was the best drug available. Yet its popularity is mostly because it was the cheapest drug at that time- other drugs cost three times as much. So it was attractive to use, especially in the developing world, and because it was available.

MS: And probably its background regarding erectile dysfunction is also a good advertisement.

GB: Yes, but it doesn’t effect patients very much in that way. Even when the drug had just been developed Pfizer didn’t advertise it for pulmonary hypertension at all- in fact, they didn’t advertise it for that for three or four years, even after the approval. They thought that this was a small altruistic treatment, not the one that was important, which was erectile dysfunction.

MS: To reiterate for the readers: you should believe in your idea, you should be brave. Correct?

GB: I am retired now, so my advice to the young people is that they have the better ideas and the most creative ideas. This is why I believe in you and in young people. Because to progress science, you must always think out of the box and outside of the doctrine that you have been taught. Science is a fact that we believe in now, but it is not the only- or the whole- truth. So sometimes you have to deviate. Unfortunately, the way the educational system is currently set up means that we indoctrinate people in one direction. So sometimes a young undergraduate can be more creative than a post-doc, because the post-doc has been programmed, and if you become a reader or a professor or a lecturer, you can sometimes only see in one direction. So often the young people are the only ones who can generate the new ideas- and if you have a good mentor, who accepts it and is courageous enough to follow through, that’s excellent. That is number one. But you don’t only need the creativity, you also need the determination. You will do it, even if people say it doesn’t work or it’s rubbish- as long as that isn’t fact, don’t let them dismiss you. This pertains not just to the story about sildenafil discovery- it’s also about the PVRI. People said the PVRI wouldn’t work- many people doubted that it could. And the journal, Pulmonary Circulation- again people said it would not work, and they said it was a crazy idea. But it exists due to the determination of the people who make things. So for the young scientists, to excel in your science, you need two things; 1. to be creative, to think outside of the box, and 2. to be determined to follow through.

Yesterday Prof. Magdi Yacoub told me a story about a successful man, a very well-known person. He is a millionaire, billionaire even. He was
was asked, “what is the secret to your success?” And he answered, “very simple. Follow up everything you are doing.”

That’s another form of determination. If you start something, don’t file it- keep doing it, and improve, and improve more. It’s the determination, follow-up, and creation of ideas- that’s the only recipe for success for young people, in science and all other things in life.

MS: Fantastic advice from a successful man. With the final question I will touch on the 8th PVRI Annual World Congress. This year it’s in China, in Guangzhou, so the eastern and western world meet here. What do you think is the advantage to different cultures coming together?

GB: Many things. First of all, the PVRI is a global organization, and we also concentrate on the developing world, so we need to engage them more and more. When you set up a meeting, a lot of the local people attend- and many of them may not have the chance to travel and visit many other meetings, so it’s a great opportunity for them to network and engage. We want the other countries to come and appreciate the abilities and benefits of this host country, which is beneficial for both. That’s the idea behind expanding and exploring- don’t stay in one place all the time.

For example, you know that some organizations only do the meeting in one place, or three places maybe, which is generally due to logistic reasons. They are too big to go just anywhere and instead need to find a place that can accommodate them. But the PVRI needs to be in all places. I take it from my own experience, because my vision to the world has changed due to the large amount of travel I used to do for my work. I started to see that the world has all the same intellectual activity, the same power- countries and cultures enrich each other. If you only live in one place, you think the rest of the world doesn’t understand anything.

So when PVRI members and fellows and groups travel and meet people, the local people will benefit and so will the PVRI delegates, as they begin to understand the need for other countries. Because the most important thing for pulmonary hypertension right now is increased awareness on a global scale. So, keep travelling from east and west all the time- go north and south too. Don’t always go back to the same place, and dare to go far away at times.

A lot of people complained when the PVRI decided to come to China, because it’s a long way for most of our delegates- most will have spent at least 15 hours travelling, and others may even have had a 50 hour journey. But- it’s worth it.

MS: Yes, and it works both ways. It’s similarly attractive for the people who always attend to see new places, people etc.

GB: Yes, and for them to learn about the various issues and interact with the locals. I mean, all the young Chinese students here- a lot of them may not have the opportunity to travel because travel is not easy, especially nowadays with the expenses and things like that. So learn from them while you are here.

MS: So Prof. Butrous, I thank you very much for this interview, it was very nice.

GB: Okay, my pleasure. Thank you.
This interviewed was transcribed by Nikki Krol, former PVRI Executive Administrator and Executive Editor of the PVRI.

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Is dietary L-carnitine a strategy to combat COPD-induced muscle wasting?

by Robert Ringseis

Prelude

Loss of skeletal muscle mass, also known as muscle wasting, is a common symptom of several chronic diseases, such as cancer, infectious diseases, and chronic obstructive pulmonary disease (COPD). Due to the strong negative impact of muscle loss on patients’ prognosis and quality of life, the development of efficacious treatment approaches to combat muscle wasting are of great importance. Interestingly, several clinical studies demonstrated that supplementation of L-carnitine (LC) has beneficial effect in patients with chronic diseases associated with muscle wasting. LC is a water soluble quaternary amine (3-hydroxy-4-N,N,N-trimethylaminobutyric acid) which is essential for normal function of all tissues with the most documented function being the translocation of long-chain fatty acids from the cytosol into the mitochondrial matrix for subsequent β-oxidation. LC in the body originates from both dietary sources (meat and dairy products) and biosynthesis in liver and kidney. LC biosynthesis probably contributes most to the whole body LC pool. This can be deduced from the observation that plasma LC levels in vegetarians, which take up only negligible amounts of LC through the diet, are only 15-30% lower than those in nonvegetarians, being yet within the normal physiological range of 25-50 µM. The plasma LC levels in vegetarians can be maintained within the normal range because they have a more efficient renal reabsorption of LC and a greater rate of LC biosynthesis. In vegetarians, LC deficiency may develop only if certain micronutrients, such as ascorbic acid, pyridoxine and iron, required as co-factors for LC biosynthesis are not provided from the diet in sufficient amounts. Noteworthy, LC was found to improve quality of life measures, nutritional status and body condition, to reduce fatigue-related symptoms and to decrease markers of oxidative stress and inflammation in patients with chronic diseases such as cancer, chronic kidney disease (CKD), HIV or hepatic encephalopathy [1-5]. In addition, a recent experimental study showed that LC reduces proteolysis in skeletal muscle, increases muscle weights and improves parameters of physical performance in tumour-bearing rats [6]. Whether LC is also effective as an anti-wasting agent in COPD patients is currently unknown. The following discussion summarizes results from animal and clinical studies showing beneficial effects of supplementation with LC or LC derivatives (acetyl-LC, propionyl-LC) on critical mechanisms involved in skeletal muscle loss under pathologic conditions, such as increased proteolysis, impaired protein synthesis, myonuclear apoptosis, inflammation, oxidative stress, and mitochondrial dysfunction. Finally, this article aims to provoke the community to think about a possible beneficial effect of LC supplementation in COPD patients.
Evidence for Inhibition of Protein Degradation

Recently, LC was found to cause a down-regulation of critical components of the ubiquitin proteasome system (UPS), the most important cellular system of protein breakdown, in gastrocnemius muscle of tumour-bearing rats [6]. This effect was accompanied by an inhibition of tumour-induced muscle wasting and an improvement of physical performance. Also in healthy animals, it was found that LC decreases gene expression of critical UPS components in skeletal muscle [7]. This effect of LC has been attributed to a stimulatory effect on the insulin like growth factor (IGF) axis [8,9]. In patients with endstage CKD it was observed that LC causes lower rates of leucine oxidation and whole-body leucine appearance from proteolysis during euglycemic hyperinsulinemic clamp conditions [10]. This indicates that LC has protein-sparing effects. In another clinical trial it was found that LC attenuates post-stress metabolism in surgical patients [11].

Evidence for Stimulation of Protein Synthesis

In rats and piglets which received total parenteral nutrition (TPN) containing LC, a greater nitrogen balance was observed [12,13], effects that were ascribed to increase energy production from fatty acid oxidation thereby causing a sparing of protein mass. In rats under unloading conditions it was found that acetyl-LC has a hypertrophic effect on type I fibers and favors a slow-oxidative phenotype of soleus muscle [14]. Likewise, Vescovo et al. [15] observed in a rat model of chronic heart failure (CHF) that LC causes an increase in the cross-sectional area of tibialis anterior muscle. In this context, Moriggi et al. [16] observed in rats under unloading conditions that acetyl-LC counteracts the unloading-induced change in muscle phenotype (shift from slow-oxidative to fast-glycolytic). This suggests that LC is able to prevent the metabolic shift from oxidative to glycolytic skeletal muscle in unloaded animals. In humans, direct parameters of protein synthesis have not been assessed in response to dietary carnitine. However, two clinical studies reported that supplementation of either LC or acetyl-LC increases serum concentrations of IGF1 in β-thalassaemic patients and asymptomatic HIV patients, respectively [17,18]. Bellinghieri et al. [19] reported that LC reduces muscle cramps in a group of uremic patients on intermittent hemodialysis (HD). Noteworthy, two previous studies reported trophic effects of LC on skeletal muscle in CKD patients undergoing HD [20,21].

Evidence for Inhibition of Apoptosis

In a rat model of monocrotaline-induced CHF, LC reduced markers of apoptosis in tibialis anterior muscle [15]. An inhibitory effect of LC on staurosporine-triggered apoptosis was also observed in cultured mouse C2C12 myotubes indicating that the anti-apoptotic effect of LC on skeletal muscle is a direct effect on myofibers. In addition, LC was demonstrated to inhibit apoptosis in gastrocnemius muscle in a genetic mouse model of human amyotrophic lateral sclerosis [22] and in tumour-bearing rats [6]. No clinical studies are available from the literature evaluating an effect of LC on myocyte apoptosis or signalling pathways regulating apoptosis in skeletal muscle. However, it has long been known that apoptosis of lymphocytes in HIV infection has great significance and lymphocyte apoptosis correlates with disease progression. Thus, several
clinical studies have evaluated an effect of LC on lymphocyte apoptosis in HIV patients [17,23,24]. Indeed, in three studies LC was followed by a significant reduction of lymphocyte apoptosis in HIV-infected patients. Indirect proof for an anti-apoptotic effect of LC was provided from two other studies in HIV patients in showing that LC increases proliferation of peripheral blood mononuclear cells and/or elevates the frequency of lymphocytes entering the S and G2-M phases of the cell cycle following mitogen stimulation [25,26].

Evidence for Inhibition of Inflammation

Animal studies consistently showed that LC exerts anti-inflammatory effects under pathologic conditions. In rats LC prevented the increase in serum tumor necrosis factor (TNF)-α levels induced by methotrexate [27]. In addition, LC produced a significant decrease in serum TNFα levels in a rat model of CHF which was associated with an inhibition of CHF-induced myopathy and skeletal muscle apoptosis [15]. Moreover, in a rat model of hypertension chronic administration of LC attenuated the inflammatory process associated with arterial hypertension [28]. Furthermore, LC was found to decrease plasma levels of cytokines in methylcholanthrene-induced sarcoma-bearing rats indicating that carnitine may ameliorate cancer cachexia through attenuating tumour-associated inflammation [29]. Also in a rat model of acute renal failure, LC was found to exert an anti-inflammatory action [30]. Using a mouse model of cancer cachexia, Liu et al. [31] observed that LC supplementation decreases plasma levels of TNFα and interleukin (IL)-6, again suggesting that carnitine inhibits cancer cachexia by reducing serum levels of cytokines which are considered as key factors for protein catabolism [32]. LC appears to be beneficial in humans as well. In a study with AIDS patients undergoing antiviral therapy LC caused a significant reduction of circulating levels of TNFα [25]. In addition, LC caused a significant reduction in plasma levels of C-reactive protein (CRP) and TNFα in patients with nonalcoholic steatohepatitis [33]. A strong anti-inflammatory effect of LC was also observed in (non-cancer) patients undergoing surgery indicating that LC protects surgical patients against surgery-induced systemic inflammation [34]. Moreover, LC causes anti-inflammatory effects in HD patients [35,36]. Interestingly, in some of these studies LC supplementation was accompanied by increases in body mass index and serum albumin levels indicating that LC supplementation improves nutritional status and causes an anti-wasting effect.

Evidence for Prevention of Oxidative Stress

Several lines of evidence from animal experiments exist that LC is effective in preventing oxidative stress under various pathological conditions. Dutta et al. [37] reported significant reductions in markers of oxidative stress and an increase in creatine kinase activity (an indirect marker of muscle damage) in gastrocnemius muscle and improvements of several indicators of muscle performance in LC-supplemented rats subjected to intermittent hypoxia. In another study, LC was effective in alleviating fructose-induced oxidative stress in rats [38]. In addition, LC suppressed oxidative modification of proteins in the hind limb muscle in a transgenic mouse model of amyotrophic lateral sclerosis [22]. Furthermore, Breitkreutz et al. [39] showed higher intramuscular levels of glutathione in tumor-bearing mice treated with LC suggesting a LC-mediated
improvement of the muscular antioxidant status in malignant diseases. Apart from this, a great number of animal studies consistently showed an anti-oxidative potential of LC in non-muscle tissues of different animal models of oxidative stress [40-44]. In humans, LC was shown to be effective in attenuating oxidative stress responses in two studies with either HIV patients [45] or CKD patients undergoing HD [46]. Moreover, in a double-blinded, placebo-controlled study with type 2 diabetic patients LC caused a significant reduction in the levels of oxidative stress markers [47]. Supplementation of LC immediately before surgical intervention was also found to be helpful in preventing major abdominal surgery-induced oxidative stress as assessed by platelet reactive oxygen species (ROS) production [48].

Evidence for Amelioration of Mitochondrial Function

An effect of LC on mitochondrial function was investigated in an experimental model of traumatic spinal cord injury (SCI), which is characterized by extensive tissue damage and mitochondrial dysfunction. Patel et al. [49] found that administration of acetyl-LC after thoracic SCI significantly maintained mitochondrial function at the injury site and daily acetyl-LC treatment increased spinal cord tissue sparing. In another study with a more severe contusion SCI model, acetyl-LC treatment resulted in significant improvements in acute mitochondrial bioenergetics and long-term hind limb function indicating that acetyl-LC promotes neuroprotection by preventing mitochondrial dysfunction [50]. Also in a mouse model of high fat diet-induced obesity propionyl-LC improved mitochondrial function [51]. In a rat model of severe cardiac hypertrophy, propionyl-LC restored the degree of reduction of mitochondrial pyridine nucleotides and improved the kinetics of mitochondrial ATP production in volume-overloaded hearts [52]. Likewise, propionyl-LC was effective in improving mitochondrial respiration and ATP production in a rat model of cardiotoxicity in which mitochondrial function is also impaired [53]. Only few studies have been conducted to explore an effect of LC on mitochondrial function in subjects with wasting-associated chronic diseases. In one study from Milazzo et al. [54] it was reported that acetyl-LC increased both 13CO2-exhalation and cumulative 13CO2 excretion as well as mitochondrial DNA content of CD4+ T-cells in HIV patients with antiretroviral therapy-related lipoatrophy indicating a protective role of acetyl-LC on mitochondrial function. A protective effect of acetyl-LC on mitochondria was also proposed by Cossarizza et al. [24] based on their finding that significantly less cells with depolarized mitochondria were found following incubation of peripheral blood lymphocytes from HIV patients with acetyl-LC.

Summary and the Question for Interactive Discussion

Comprehensive analysis of results from both animal and clinical studies shows that LC supplementation beneficially influences several critical mechanisms involved in skeletal muscle loss under pathologic conditions, such as increasing protein synthesis, reducing protein degradation, inhibiting apoptosis, abrogating inflammation, preventing oxidative stress and ameliorating mitochondrial function. Based on the fact that similar mechanisms are known to be involved in COPD, we would like to postulate the following question: Could supplementation with
LC serve as an effective anti-wasting approach for patients with COPD?

From our point of view, COPD patients might benefit from LC supplementation because LC status is frequently impaired, e.g. plasma LC levels are reduced, in chronically ill patients [55-57] as a consequence of a reduced food intake, which is accompanied by a decreased dietary uptake of LC and specific micronutrients required as co-factors for LC synthesis. In addition, it has been reported that pharmacotherapy in chronically ill patients often causes an impaired LC status as a side effect due to a reduction of LC absorption from the intestine and/or a stimulation of urinary LC excretion [58,59]. A reduction of intestinal LC absorption might be relevant in COPD patients undergoing treatment with the anticholinergic drug ipratopium due to competition for the same transport mechanism in the intestine, namely the active transport by the sodium-dependent novel organic cation transporters OCTN1 and OCNT2. Indeed, ipratopium was reported to be a strong inhibitor of OCTN2-mediated LC uptake [60]. Irrespective of the efficacy of dietary LC supplementation as an anti-wasting approach in COPD patients, it is worth to mention that none of the clinical studies dealing with LC supplementation reported any adverse effects even at very high dosages, at least in the short-term. However, with regard to possible adverse effects of long-term LC supplementation, one recent study has to be mentioned showing that chronic dietary LC supplementation in mice alters cecal microbial composition, markedly enhances synthesis of trimethylamine (TMA) and the proatherogenic species trimethylamine-N-oxide (TMAO), and increases atherosclerosis [61]. Moreover, these authors reported that omnivorous subjects produce significantly more TMAO than vegans/vegetarians following ingestion of LC through a microbiota-dependent mechanism. In light of these recent findings safety aspects of chronic LC supplementation have to be critically evaluated.

Scientists and other persons who are interested in this field are invited to express their views, pro or contra, about this topic in the next issue of PVRI Chronicle.

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Dysregulated inflammatory response in pulmonary hypertension is still enigmatic: what about psoriasin?

by Djuro Kosanovic

Prelude

Altered regulation of the immune system and enhanced inflammation were recognized as important characteristics of the pulmonary hypertension (PH) pathology. However, many underlying molecular mechanisms responsible for such uncontrolled inflammatory response in this incurable pulmonary vascular disease remained unclear and need to be further enlighten. Psoriasin (S100A7), a molecular mediator discovered over two decades ago in the pathology of psoriasis, plays a significant role in innate immunity and antimicrobial battle, skin inflammation and tumor development and progression. Due to its potent pro-inflammatory power one can expect that psoriasin may represent a novel culprit involved in the PH pathogenesis. However, up to date nothing is known regarding its potential role in the context of pulmonary vascular disease.

Main Article

Despite its beneficial role as a part of the immune system defense against microorganisms, overexpression of psoriasin was found to be responsible for increased tumor growth and metastasis via orchestration of various inflammatory and pro-proliferative pathways/mediators and events, such as increased CCL2 (chemokine (C-C motif) ligand 2)/MCP-1, MMP-9 and abnormal accumulation of macrophages are already described, suggesting the hypothetical link between psoriasin and pulmonary vascular disease (Figure 1). Furthermore, it was demonstrated that psoriasin stimulated neutrophil-associated production of different cytokines and chemokines, particularly IL-6 (interleukin-6) and TNF-α (tumor necrosis factor-α). IL-6 and TNF-α are important culprits for the PH development, indicating another theoretical possibility how psoriasin could contribute to the pathology of the pulmonary circulation (Figure 1). Shifting from the cancer field to the lungs and respiratory disorders, psoriasin was found to be expressed in the lung epithelial cells and macrophages. Interestingly, psoriasin profile did not change in the patients with chronic obstructive pulmonary disease, compared to the healthy controls. Surprisingly, one has also to keep in mind the existence of potentially anti-fibrotic effects of psoriasin. Finally, the literature suggested that reactive oxygen species (ROS), an important feature of the PH pathobiology, are also described as inducers of psoriasin. Even more, psoriasin itself was shown to induce ROS production in epithelial and endothelial cells, indicating the probable existence of the positive loop between ROS and psoriasin. In addition, it was found that psoriasin acting via receptor for advanced glycation end products (RAGE) leads to augmented proliferation of endothelial cells and further increase of ROS. Following this line of thinking, it is worth to mention that dysregulated endothelial cells and injured endothelium are indeed involved in the complex PH pathology, so one can hypothesize that similar effects of psoriasin may be possible in the context of the pulmonary vascular endothelium (Figure 1).
In conclusion, we propose that psoriasin, due to its potent pro-inflammatory and pro-proliferative properties acts as a molecular conductor in orchestration of different signals and events, such as ROS, inflammatory cells accumulation, cytokines, chemokines etc. in the pathological opera of the pulmonary vascular remodeling (Figure 1).

**The Question for Interactive Discussion:**

We would like to suggest the following question to the scientific community worldwide: Is psoriasin a potential mediator with properties to be involved in aggravation of already altered inflammation in the pathobiology of pulmonary hypertension? All experts and others interested in this field are welcome to reply and express their point of view and perspectives on this topic, in the next volume of PVRI Chronicle.
References


Abnormal right ventricular relaxation in pulmonary hypertension

**Introduction:** A common complication of systemic hypertension is left ventricular diastolic dysfunction. Sparse evidence is available of a similar phenomenon occurring in the right ventricle.

**Hypothesis:** Pulmonary hypertension (PHT) is associated with abnormal right ventricle (RV) early relaxation time.

**Methods**
- RV pressure measured by a high fidelity catheter in 25 patients with and without PHT
- Diastolic measures:
  - Min diastolic pressure (dRVP)
  - End-diastolic pressure (RVEDP)
  - Early relaxation (RV tau)
- Systolic measures:
  - End systolic elastance (Ees)

**Diastolic function**
- Increased diastolic and end-diastolic pressure

**Systolic function**
- Preserved coupling ratios (Ees/Ea)
  - RV contractility (Ees) tended to increase with increased arterial afterload (Ea)

**Conclusion:** Abnormal measures of RV early relaxation with static or increased RV contractility suggests RV diastolic dysfunction may proceed systolic dysfunction.

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Non-communicable diseases (NCD) account for more than 65% of worldwide deaths, and are determined by a mix of environmental, genetic and epigenetic factors. (1). One important NCD is pulmonary hypertension that may be triggered by environmental hypoxia. An increased risk of developing cardiovascular and pulmonary diseases has been linked with in utero adverse conditions such as chronic hypoxia (2-5). Furthermore, hypoxia in postnatal life determines cardiovascular and pulmonary responses that may turn mal-adaptive and induce chronic disease (6-8). All of the above are particularly relevant in human populations exposed to high altitude, either in chronic continuous (permanent inhabitants) or intermittent fashion (high altitude workers, mountaineers and tourists). Worldwide, it is estimated that over 140 million people live in altitudes above 2,500 m. (9). The densest populations above 3,000 m. are in the South American Andean Mountains (9). In Central and South America alone, it is estimated that more than 35,000,000 people live at high-lands (10) and in Chile more than 30,000 work in high-altitude shifts (11).

The Andean Altiplano, or Alto Andino, is a ~4,000 m. high plateau located in the Central Andean Range. It has two types of human population: a permanent one consisting mainly of quechua and aymara groups (pure and caucasic-mixed), and an intermittent one composed of mining workers and staff, tourists, mountaineers, military soldiers and customs workers. The area is rich in endemic and introduced animals permanently living under hypobaric hypoxia. All of these characteristics offer an exceptional opportunity for the study of humans and animals exposed to chronic or intermittent hypobaric hypoxia. Therefore, the Altiplano becomes a fundamental natural laboratory, where unique scientific data can be obtained from species adapted or mal-adapted to chronic hypoxia.

Currently, the Andean plateau enjoys good ground and air travelling accessibility. Given this, University of Chile created the International Center for Andean Studies (INCAS) in the early 2000s in Putre (at 3,600 m), a town located in the very north of Chile and adjacent to Peruvian and Bolivian borders. Since then, several researchers have been working on animal and human cardiovascular and pulmonary adaptations to high altitude in this Center. Dr. Anibal J. Llanos, as one of the founders of this Center, developed a pulmonary hypertensive lamb model (Ovis aries) (12-16). He and his group have extensively characterized this non-adapted neonatal model, and compared the pulmonary physiology with the llama (Lama glama), a high-altitude adapted mammal gestated, born and raised above 4,000 m. with no pulmonary hypertension, offering exclusive evolutionary selections to cope with high-altitude (14,17-23).

The Putre Research Station-INCAS is open all year long to be used by Chilean and foreign researchers interested in any scientific discipline related to high altitude environments. The Center has housed research projects in diverse areas such as physiological and molecular adaptations of the pulmonary circulation to hypoxia in fetus, newborns and adults in animals raised at high-altitude. The Station has separated house and laboratory facilities, which can lodge comfortably 4 to 6
researchers (Figure 1).

Figure 1 Facilities of the International Center for Andean Studies (INCAS). The picture show an expedition by the authors of this manuscript. At the back is the housing area that can easily accommodate 4-6 persons. Further there are several hotels and hostels in town.

It is located in Putre, the capital of the Chilean Andean region of Arica and Parinacota, 140 km from Arica at sea level connected by a paved road. This same road can take you in only 5 h to the capital of Bolivia, La Paz, at 3,600 m (Figure 2).

Figure 2. Location of the International Center for Andean Studies (INCAS). The research station is located in Putre, at 3,600 m. in the north of Chile with expeditious accesses.

Putre has access to basic services such as surgery-house, school, restaurants, hotels and several shops.

Several studies have shown that hypoxia at high altitude should be considered a health risk during development and adulthood (24-25) and it should be taken as a public health issue. Hence, establishing the impact of oxygen restriction throughout the lifespan represent a substantial advantage in understanding the role of hypoxia in determining cardiopulmonary diseases in the highlands. Scientists must access high-altitude areas to be able to understand the physiology and pathophysiology under chronic hypoxia. The INCAS research station is available to serve this need as a unique natural Laboratory of hypobaric hypoxia.

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Did you know...the fluid extravasation which leads to pulmonary edema may occur across large vessels as well as capillaries?

by Kevin Lowe

Figure 1. Two sites of fluid accumulation. Increased capillary permeability leads to alveolar flooding (left panel). Increased artery and/or vein permeability leads to perivascular cuffing (right panel).

Did you know...the fluid extravasation which leads to pulmonary edema may occur across large vessels as well as capillaries?

Due to the large surface area of the capillary network, all extra-vascular pulmonary fluid has traditionally been thought to result from pathological fluid movement across the capillary endothelium. In this model, fluid leaking from capillaries is drawn by negative interstitial pressure to the extra-alveolar interstitium, and moves into alveoli only when interstitial pressure reaches some critical value which forces fluid across the alveolar epithelium [1]. However, there is considerable evidence from animal models to suggest that extra-vascular pulmonary fluid may result from fluid flux across arteries or veins as well as capillaries. Rat pulmonary venules are more permeable than capillaries when edema is induced with alpha-naphthylthiourea [2]. Arteries have been shown to be the primary source of extra-vascular fluid in models of hypoxic pulmonary edema [3]. Under conditions of increased vascular pressure in excised or in situ dog lungs, fluid movement across extra-alveolar vessels accounts for approximately 60% of extra-vascular fluid accumulation [4, 5]. In rat and rabbit models of acute lung injury, 50 to 75% of vascular permeability occurs in extra-alveolar vessels [6, 7]. Treatment of isolated lungs with the plant alkaloid thapsigargin induces increased permeability in large pulmonary vessels, due to retraction of cell-cell borders [8].

Of course, fluid filling of alveoli does occur during fulminate pulmonary edema and likely has significant pathophysiologic effects. For this reason, research has focused on the mechanisms controlling the accumulation and removal of fluid in the alveolar airspace [9]. However, fluid accumulation outside the alveolar airspace has also been documented in both human pathology and in animal models. Extra-alveolar fluid accumulates in the interstitium surrounding large pulmonary vessels and is often termed ‘vascular cuffing’ (see Figure). Such fluid collections have been described in lungs of patients and animals with sepsis due to Pseudomonas aeruginosa [10]. Extra-alveolar fluid collections also likely occur in patients with edema due to heart failure [11], and are documented in numerous animal models of pulmonary edema [4, 12]. Researchers have suggested that such extra-alveolar fluid accumulation contributes to airway narrowing and increased resistance to airflow [13], increased airway closing pressure and functional residual capacity in post myocardial infarct patients [11], and increased pulmonary vascular resistance [14].

In summary, classic models of fluid movement within the lung suggest that pulmonary edema results when fluid crosses the capillary membrane. However, evidence that extra-alveolar vessels may be more permeable than capillaries under both normal and pathological conditions should lead us to question the ubiquitous application of this model. Future research into the pathophysiology of diseases like Acute Respiratory Distress Syndrome (ARDS) will require accurate descriptions of the sites along the arterial-capillary-venous axis where increases in permeability occur.
References


Advances in understanding of pulmonary arterial hypertension and the evolution of Experimental pulmonary hypertension models

by Michiel Alexander de Raaf

“Primary Pulmonary Hypertension has been called the cardiologist’s cancer”

Greg Elliott in personal conversation with Norbert Voelkel, early 80’s

Pulmonary Arterial Hypertension (PAH) can be a rapidly progressive and devastating disease characterized by dysfunction and remodeling of the pulmonary vasculature, leading to increased pulmonary vascular resistance. The increased vascular resistance pushes the right ventricle (RV) into adaptive compensatory remodeling by hypertrophy, but eventually RV dilatation, heart failure and death of the patient become inevitable [1, 2]. Today, 3 pathways are targeted in PAH treatment: the nitric oxide-cyclic guanosine monophosphate pathway, the endothelin pathway and the prostacyclin pathway [3]. Although treatments affecting these pathways delay disease progression and increase survival rates [3, 4], they do not cure PAH [3]. The pathogenic paradigm of PAH has shifted from pulmonary vasoconstriction driven by smooth muscle cells to vascular remodeling affecting all vessel wall layers. In this chapter, a brief overview will be given of how PAH research has converged on the role of the endothelial cell (EC). It will be argued that the development of new animal models has been critical to the development of a new PAH paradigm.

In 1970, the first morphologic characterization of the pulmonary hypertensive lung was published by Wagenvoort et al. [5, 6], followed by the first WHO classification of Pulmonary Hypertension in 1972. In this classification, the pathological focus in PAH, then called Primary Pulmonary Hypertension, was on the vascular media of the pulmonary arterioles, featuring hypertrophied and hyperplastic pulmonary artery smooth muscle cells (PASMC) [1, 2, 7]. The “vascular media paradigm” of PAH ascribed a major pathogenic role to sustained pulmonary vasoconstriction and was fueled by two PAH outbreaks related to the use of the appetite suppressants Aminorex (Menocil*) and fenfluramine (Ponderal*) in the late 60’s and 80’s. Both drugs are serotonin transporter substrates acting on the PASMC and promote vasoconstriction and PASMC hypertrophy and proliferation. At that time, the signature plexiform lesion was regarded as an epiphenomenon.

The description of idiopathic PAH as ‘the cardiologist’s cancer’ in the early 80’s meant at that time PAH and cancer shared a common clinical and scientific context. Both conditions were untreatable due to a profound lack of understanding and absence of technologies to study pathogenesis and pathobiology. As dedicated research did not lead to a medical cure for PAH, the idea that other vascular wall cells than smooth muscle cells were also involved in the disease was gradually accepted. For a better understanding of PAH, it was necessary to study the cellular and molecular aspects of the disease. An expanding research effort revealed the mechanisms by which PAH is characterized today: endothelial dysfunction, PASMC hypertrophy and hyperplasia, persistent inflammation and dysimmunity and dysregulated intra- and extracellular cell signaling in the cells of all layers of the pulmonary vessel bed, leading to pulmonary vessel remodeling [8–10]. Endothelial dysfunction is reflected in increased activity of contractive
vasoactive agents as endothelin-1, serotonin, angiotensin II and decreased activity of dilative vasoactive agents as nitric oxide and prostacyclin, felt to lead to persistent vasoconstriction. The hyperproliferative endothelium in PAH disobeys the “law-of-the-monolayer” and has a monoclonal origin in plexiform lesions [11–13]. This hyperproliferation is mediated by increased activity of growth factors as fibroblast growth factor-2, platelet derived growth factor and epidermal growth factor and is accompanied by apoptopic resistance [14–17]. Also in the PASMC the increased activity of growth factors and apoptosis resistance are observed. As well in the EC, hyperproliferation of PASMC is mediated by migration and dysregulated BMPRII signaling, for example due to the germline BMPRII-mutation, which has a penetrance of approximately 75% in hereditary familial PAH [10, 14, 15]. Another germline mutation in PAH is found in the KNCK3 gene, which decreases the activity of TWIK-related acid-sensitive K+ channel-1 [15]. Together with alterations in the voltage-gated K+ channel, transient receptor potential 1 and 6 and calcium sensing receptor, the PASMC is hyperpolarized [9, 18, 19]. Moreover, the PASMC in PAH goes through an energetic shift to glycolysis [20], also found in fibroblast of the pulmonary adventitia [21], which is similar to the Warburg effect. These dysregulated mechanisms also affect the extracellular matrix [22], and contribute to the abnormal cell signaling leading to disordered angiogenesis [16, 17, 23], and recruit inflammatory cells in the pulmonary vasculature which can release cytokines and chemokines as for example interleukin 1 and 6 [11, 15, 24–26]. It is now recognized that all layers of the vessel wall contribute to the pulmonary vascular remodeling; including the hyperproliferative endothelium, and the plexiform lesion is no longer neglected as a hallmark of PAH [27, 28]. Many of the observations made during the study of pulmonary vascular remodeling in PAH, echoed the description of the hallmarks of cancer as described by Hanahan and Weinberg [28–31]. This expansion in knowledge gave rise to the exploration of potential treatment targets, which were already studied for their efficacy in cancer, such as Histone DeACetylate inhibitors (HDACis) and Tyrosine Kinase Inhibitors (TKIs) [32].

**Experimental models to resemble pulmonary arterial hypertension**

Translational research on the development of pulmonary hypertension and potential treatments of the disease has relied on animal models that replicate one or more important aspects of the disease [33–36]. Reeves and Grover were the first to use hypoxia induced pulmonary hypertension as a model of the human condition when they studied Brisket disease in cattle kept at high altitude [37, 38]. Exposure to hypoxia was soon also used to study pulmonary hypertension in rats. In addition, an animal model based on the administration of monocrotaline was first used in 1967 [39, 40]. The monocrotaline and chronic hypoxia animal models of pulmonary hypertension both exhibit profound remodeling of the vascular media and hypertrophy of PASMC. Because these phenomena were considered singularly important in human pulmonary hypertension, it was felt that there was little need to develop alternative animal models.

The expansion in pathobiological knowledge of PAH has coincided with the establishment of new animal models of the disease. When in 2001 the Sugen Hypoxia model (SuHx) of PAH was...
discovered [41], the striking resemblance was appreciated between the pathological changes in the SuHx rat and the human PAH lung. More specifically, it was noted that the intima remodeling and obliterator vascular remodeling that is typical of human PAH, was mimicked by the lung vascular changes found in the SuHx rat [35, 41, 42]. The SuHx model also brought the understanding that the emergence of a proliferative endothelium could require an initial phase of endothelial apoptosis, as it could be prevented by a broad spectrum caspase inhibitor [41, 43]. Also in other new animal models intimal proliferation were found in the combination of monocrotaline plus aortic caval shunt or monocrotaline plus pneumonectomy [44–46].

Despite increasing popularity, the SuHx model has not (yet) become a ‘gold standard’ for animal research in PAH. In comparison to the classic animal models, the SuHx model is more complex, has not been thoroughly characterized and is more difficult to implement and harmonize among laboratories. In addition, a vast amount of translational studies has been performed with the chronic hypoxia and monocrotaline models and this body of work is a heritage of historical literature to which new data is conveniently compared. Therefore, many translational studies are still performed using the traditional animal models. Remarkably, many potential treatments adopted from the cancer field, are still explored in the classic animal models that do not represent the pathobiology of angio-obliterator PAH nor offer a hyperproliferative endothelium as a treatment target. This might explain why therapeutic interventions seem curative in animal studies whereas human PAH remains refractory to treatment [47, 48].

**Conclusion**

Due to the ineffectiveness of vasodilating drugs and aided by many cellular and molecular findings, the pathogenic paradigm of PAH has shifted from vasoconstriction towards vessel remodeling with resemblances to malignancy. Concurrently, novel animal models have evolved, which feature the human hallmark of PAH, a hyperproliferative endothelium, that is not found in more traditional animal models. Therefore, when these traditional animal models are used to assess anti-proliferative drug targets of the endothelium, they become unpredictable in their translational value. Of course, due to the unknown pathogenesis of PAH, it is paradoxically true that no animal model can resemble PAH completely. However, with the increasing realization and evaluation of the different pathobiologies resembled by animal models [33–35], the selection of the used animal model should be explicitly motivated.
# References


A 22-year-old female with positive antiphospholipid antibodies was referred for pulmonary hypertension (PH) evaluation. She had history of deep venous thrombosis associated with ankle fracture when she was a teenager. She was on chronic warfarin therapy with target INR of 2.5-4.0. Patient complaint of progressive worsening of shortness of breath over a year and worsening dyspnea on exertion. Ventilation-perfusion scan done as part of PH work up revealed a high probability scan. This patient underwent right heart catheterization in July 2009 and her hemodynamics were as follows: RA mean 4 mm Hg, PA pressure 96/36 mm Hg (mean 57), PCW 17 mm Hg and step up consistent with left to right shunt. Subsequent cardiac magnetic resolution imaging done to evaluate her “step up” showed no atrial septal defect, ventricular septal defect or anomalous pulmonary venous return. She was started on sildenafil. The patient underwent pulmonary arteriography on August 2010 that revealed “pouch web in the LUL, narrowing of the left descending PA, vessel attenuation in LLL, with narrowing of the right intra-lobar artery. These results were suggestive of lining disease of the descending pulmonary artery, concerning for pulmonary artery sarcoma. She was diagnosed with chronic thromboembolic pulmonary hypertension (CTEPH). Due to the poor prognosis and short life expectancy of this potential diagnosis, she underwent bilateral pulmonary thromboendarterectomy (PTE) surgery in March 2011 with removal of multiple emboli. No evidence of tumor was noted. The patient experienced significant improvement in her symptoms including resolution of her shortness of breath. The post-operative hemodynamics were: PA 50/22 mm Hg (mean 34) and CVP 15 mm Hg. Although this patient had persistent post-thromboendarterectomy PH, the postoperative course was uncomplicated. The patient remained on warfarin with higher INR goals for positive antiphospholipid antibodies but also due to the presence of small distal clots that were noted during PTE.

Chronic thromboembolic disease is an under diagnosed and under treated disease. There are about 600,000 cases per year of acute pulmonary embolism (PE) in United States of America. Estimated incidence of chronic thromboembolic pulmonary hypertension after acute PE is about 0.5% to over 4%. Based on a registry (2007 to 2009) including 679 patients from 16 European countries and Canada, history of acute PE was reported in nearly 75% of cases. A multi-disciplinary team evaluation to determine candidacy for endarterectomy is important. As a potentially reversible cause of pulmonary hypertension, the recommended treatment for CTEPH is PTE. Appropriate patients with proximal clot burden who undergo this surgery have a good prognosis. In experienced centers, the mortality from this surgery is less than 4% and most patient report improvement of symptoms, resolution of PH, and this surgery in many cases obviates the need for PH therapy. CTEPH should always be included in the differential in a patient undergoing PH evaluation.
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Figure: Characteristic specimens retrieved during pulmonary thromboendarterectomy surgery from a patient with CTEPH.
In February 2015, the PVRI welcomed a new member of staff to its central office in the UK, to replace Nikki Krol, PVRI Executive Administrator & Executive Editor. After four years of dedicated service, Nikki decided to leave the PVRI in order to pursue new challenges in London. Following a rigorous interview process, Aaron Shefras, a recent ‘Film, Radio and TV Studies’ graduate from Canterbury Christ Church University, UK, was appointed as the new administrative face of the PVRI. Following a trial period of two months, during which Aaron performed outstandingly, the PVRI was delighted to offer Aaron a permanent contract.

Given his marketing background and ‘eye for design’, Aaron has already made vast improvements to the PVRI’s image resulting in our first official brochure, conference flyers for both the London Symposium and Annual World Congress in Rome, an improved image of the monthly PVRI online newsletter and, of course, the new design of this journal, the PVRI Chronicle.

Further testament to his skills and hard work are the excellent e-learning videos he has produced of the 8th SAPH (Saudi Association of Pulmonary Hypertension) Conference talks and the recent London Drug Discovery & Development Symposium lectures. The video he put together of our first fundraising event – the PVRI Charity Concert which was held on 5th May 2015 in honour of Global Pulmonary Hypertension Day – received outstanding positive feedback and resulted in the most popular PVRI post on our social networks, receiving over 1,500 views. All lecture recordings, interview talks and the concert video can be viewed on the PVRI website: www.pvri.info.

Juggling numerous tasks on a daily basis, Aaron is now fully involved in the development of our new PVRI/Pulmonary Circulation website which will go live in January 2016.
Who is the PVRI?

Belief & Story

Why do we exist as an organisation?
Our belief is that we are only able to do what we do because of global collaboration.

What does collaboration mean to us?
Collaborating means trusting someone else to have more valuable input than yourself.

We create a forum for people to build this trust. Our members challenge each other, they learn from one another and the conversations they have change the shape of the medical community together.

Our members range from post-doctoral students, to the world’s leading scientists and clinicians.
This holistic perspective is absolutely unique to us as an organisation and one we are most proud of.

The result of this ethos is globally raising the profile of PVD, facilitating research of PVD and encouraging the development of new medicines.

Enforcing the belief.


We apply the beliefs of our organisation in three ways.

Inform:
We inform & educate the world about PVD through our learning materials & publications.

Collaborate:
We enable our members to collaborate through our bi-annual conferences and many national symposia.

Combat:
Our Task Forces specialise in 8 forms of the disease and we combat PVD first hand in over 60 different countries.