“Angiopoietin-Like Protein 4 Reduces Infarct Size by Preserving Vascular Integrity and Reducing No-Reflow”

Stéphane Germain, director of research, Institut National de la Santé et de la Recherche Médicale, and group leader, Centre for Interdisciplinary Research, Collège de France, Paris, France, talks to Judy Ozkan, BA.

In 2000, Stéphane Germain, BSc, PhD, director of research at the Institut National de la Santé et de la Recherche Médicale (INSERM) and group leader at the Centre for Interdisciplinary Research of the Collège de France in Paris, France, took up an intriguing challenge. During a sabbatical in Boston, MA, his previous boss, Pierre Corvol, MD, PhD (see http://circ.ahajournals.org/content/113/20/f77), who played a key role in the development of drugs acting on the renin-angiotensin system, had been inspired by the discoveries surrounding angiogenesis made by the late Judah Folkman, MD, and molecular biologist Napoleone Ferrara, MD, PhD, among others. Dr Germain explains, “There was great excitement about angiogenesis at that time, and Professor Corvol offered me an empty lab with the challenge, which was quite crazy, in retrospect, to work on angiogenesis. I accepted this rare opportunity to set up a new lab by myself in the great environment of the Collège de France and publish something new in the field within 3 years.” There was no special support or funding. Dr Germain continues, “Taking advantage of techniques I had learnt in Boston, I set up a screen aimed at identifying genes whose expression is upregulated in endothelial cells. We identified roughly 350 genes that were upregulated in endothelial cells, and some of them were unknown. This was exciting. We started by showing that the expression of the angiopoietin-like protein 4 gene (angptl4) is upregulated by hypoxia in endothelial cells in vitro and in vivo.1

“I thus fulfilled the first of my goals in identifying and characterising the function of a protein that might regulate hypoxia angiogenesis in pathological situations (ie, critical leg ischaemia and cancer). Although this work was very challenging, it was unique and very enjoyable.”

“Having Discovered That Angiopoietin-Like Protein 4 Might Protect the Coronary Vascular Network, It Clearly Deserves More Attention. One of My Goals in the Future Is to Transfer These Findings to the Clinic”

Dr Germain and his colleagues and collaborators then went on to characterise the function of genes identified from this screen to identify which steps of angiogenesis might be related to these gene products.2,3

He explains, “Increased permeability, predominantly controlled by endothelial junction stability, is an early event in the deterioration of vascular integrity in ischaemic disorders and cancer. Although primary percutaneous coronary intervention achieves epicardial coronary artery reperfusion, paradoxical abnormal myocardial perfusion persists in 5% to 50% of myocardial infarction patients despite the lack of angiographic evidence of mechanical vessel obstruction (ie, no-reflow). Reperfusion of the ischaemic myocardium can induce reperfusion injury (ie, microvascular dysfunction and leaky vessels which promote oedema and leukocyte infiltration). In these patients, the no-reflow phenomenon is a strong predictor of poor clinical outcome...
and 5-year mortality. Thus, preservation of vascular integrity is fundamental in ischaemic heart disease. We tackled this problem thanks to the skills that Ariane Galaup, PhD, learnt while in the lab of Elisabetta Dejana, PhD, at IFOM-Milan, Italy, and also from Donald McDonald, MD, PhD, at the Department of Anatomy at the University of California, San Francisco, CA. We demonstrated that angiopoietin-like protein 4, which counteracts ischaemia-induced vascular endothelial growth factor (VEGF) signalling and disruption of endothelial cell junctions, leads to subsequent protection of the coronary capillary network and reduction of infarct size. Angiopoietin-like protein 4 reduces infarct size by preserving vascular integrity and reducing no-reflow and therefore represents a promising pharmacological and therapeutic approach, either alone or in combination with other strategies, for cardioprotection during acute MI [myocardial infarction] by targeting the no-reflow phenomenon.

“The Circulation article describing this work was initiated in my lab but gained maturity through an important collaboration with Alain Berdeaux, MD, PhD, and his collaborators, Bijan Ghaleh, PhD, Sandrine Pons, PhD, Renaud Tissier, DVM, PhD, and Valérie Martin, BSc, at Creteil Medical School-INSERM U 955 equipe 3, Creteil, France. Another important collaborator is Rachid Souktani, PhD, from the Plateforme d’Exploration Fonctionnelle du Petit Animal, who participated in the initial experiments. Professors Berdeaux and Ghaleh had benefitted from post-doctoral training in the lab of Stephen Vatner MD, of Harvard Medical School, Boston, MA, gaining expertise in large animal models. The main research area of this team encompasses myocardial ischaemia (infarction, stunning cardiac arrest) and the search for cardioprotective strategies to prevent the deleterious consequences of myocardial ischaemia. Their goal is to translate concepts developed in the lab to clinical trials, and they have contributed to the initiation of 3 ongoing clinical trials to assess the effect of proposed cardioprotective strategies.

“Having discovered that angiopoietin-like protein 4 might protect the coronary vascular network, it clearly deserves more attention. One of my goals in the future is to transfer these findings to the clinic. It is a long road but it deserves to be tried.”

“I Wanted to Study Signalling Pathways to Better Appreciate the Molecular Mechanisms Responsible for Modulating Renin Gene Expression”

For his PhD, Dr Germain investigated the transcriptional regulation of the human renin gene under the supervision of Florence Pinet, PhD, in Professor Corvol’s lab in Paris, France. In 1997, Professor Corvol took a sabbatical in the lab of the late Edgar Haber, MD, director of the Centre for the Prevention of Cardiovascular Disease, Harvard University, and invited Dr Germain to visit. The visits were life-changing for Dr Germain. “It was like a trigger for me,” he recalls. “In addition to sharing a few glasses of Professor Corvol’s beloved whisky, I discovered a new environment, new people, and new techniques. At that time, there was huge excitement about differential gene expression studies, and I was interested in identifying new transcription factors that regulate renin gene expression in juxtaglomerular cells by comparing the gene expression pattern of isolated juxtaglomerular apparatuses of a clipped kidney where renin is massively expressed versus a contralateral kidney where renin expression is extinguished. This task was difficult and challenging, and although we were not completely successful, it was a great experience.”
After completing his PhD in 1998, Dr Germain altered his research focus. He says, “Previously, my studies were directed towards protein–DNA interactions. I now wanted to study signalling pathways to better appreciate the molecular mechanisms responsible for modulating renin gene expression (extracellular cues such as pressure, flow, salt, etc.). SMAD proteins had just been identified by many labs, and I was interested in the transforming growth factor-beta signalling pathway, so I contacted Professor Caroline Hill, PhD, who was setting up her own lab at the former Imperial Cancer Research Fund, now Cancer Research UK, in London, England. Caroline had an interesting theory that SMAD proteins, which were activated by transforming growth factor-beta signalling, could then interact with transcription factors and bind to regulatory regions of genes whose expression is activated by transforming growth factor-beta. The first example of this was demonstrated for FAST-1, which associates with SMAD in response to an activin/transforming growth factor-beta signal to form a complex that recognises the *Xenopus* activin responsive element, a discovery made by Malcolm Whitman, PhD, of the Department of Cell Biology at Harvard Medical School.

“I conducted experiments that led to the identification of a SMAD interaction motif that is present in transcription factors in various families, such as homeodomain and winged-helix transcription factors, which recruit activated SMADs to distinct promoter elements via this common SMAD interaction motif. This was exciting and resulted in 2 highly cited articles.”5,6

Dr Germain and his group (Dr Catherine Monnot and Dr Laurent Muller) are currently working on 3 main research goals surrounding the vascular contribution to ischaemic and tumour disease: vascular integrity in the acute phase of ischaemic diseases and the mechanisms underlying the no-reflow phenomenon; the role of extracellular matrix scaffolding in regulating angiogenesis;3 and new molecular and mechanistic roles of microRNAs in various aspects of vessel formation in collaboration with Marc Tjwa, MD, PhD, group leader, Lab of Vascular Haematology/Angiogenesis, Institute for Transfusion Medicine, Goethe University, Frankfurt, Germany.

References

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Supporting Spanish Graduates to Carry Out Research in Universities and Research Institutions Elsewhere in Europe, the United States, and Canada

Cardiovascular researchers whose work has been funded by a Caja Madrid Foundation fellowship describe the application process and the research it funded to Jennifer Taylor, BSc, MSc, MPhil.

José V. Pérez Girón, PhD, research fellow, Department of Human Physiology, Universidad Rey Juan Carlos, Madrid, Spain

Dr Pérez used a fellowship awarded in 2010 to study pulmonary angiogenesis in the unit of Anne Tsicopoulos, MD, at the Centre of Infection and Immunity of Lille, Lille, France, for 6 months and will now investigate the molecular mechanism underlying the pathogenesis of influenza A virus infection with Cesar Muñoz-Fontela, PhD, at the New Emerging Viruses Unit, Heinrich Pette Institute, Hamburg, Germany, for 17 months.

Dr Pérez’s research focuses on the vascular inflammation associated with vascular hypertension. In 2009, he received his PhD from the Universidad Rey Juan Carlos and created a nanobiotechnological enterprise for engineering biosensors to detect molecular alterations with cardiovascular relevance. “My scientific background motivates me to continue in the area of vascular inflammation but is more focused on its immunological component,” he says. “I hope not only to increase my scientific and human skills but also to acquire knowledge in new molecular techniques that could be used in the future in the cardiovascular area.”

Gorka San José Enériz, PhD, research associate, Centre for Applied Medical Research, University of Navarra, Navarra, Spain

Dr San José used a Caja Madrid Foundation fellowship awarded in 2009 to fund a 9-month postdoctoral fellowship in the Cardiovascular Division, King’s College London, headed by Professor Ajay Shah, MD (see http://circ.ahajournals.org/content/122/5/f25).

“I thought it was a good opportunity for me to grow both professionally and personally,” Dr San José says. “And I thought it was good for the lab that I worked for an international group of excellence in the heart and the NADPH [nicotinamide adenine dinucleotide phosphate] oxidase system.”

Dr San José’s research involved the role of oxidative stress in the regulation of cardiomyocyte contraction. He focused on 1 member of the NADPH oxidase family, Nox4, and compared the absence and presence of this protein in the contractile function of mouse cardiomyocytes. He was also involved in discovering a novel splice variant of Nox4. Before the fellowship, Dr San José studied the promoter of the p22phox subunit, 1 of the components of the NADPH oxidase system, first in the spontaneously hypertensive rat and then in humans.

“We found several polymorphisms in a large population of hypertensive patients that associate with essential hypertension and with an enhanced NADPH oxidase activity,” he explains. “In atherosclerosis, we found that systemic NADPH oxidase activity was associated with carotid intima media thickness and with the secretion of matrix metalloproteinase 9 in monocytes. Similarly, in metabolic syndrome and insulin resistant patients, the systemic NADPH oxidase activity from peripheral mononuclear cells was enhanced.”
Ana M. Briones Alonso, PhD, research fellow, Department of Pharmacology, Faculty of Medicine, Universidad Autónoma de Madrid, Madrid

Dr Briones used a 12-month fellowship awarded in 2008 to expand her knowledge at the molecular and cellular levels spanning basic and clinical vascular research in the lab of Professor Rhian Touyz, MD, Kidney Research Centre, Ottawa Hospital Research Institute, University of Ottawa, Ottawa, Canada. After receiving her PhD in 2000, Dr Briones completed her first postdoc at Universitat Autonoma de Barcelona. Then, back at Universidad Autónoma de Madrid, she worked for 2 short terms in Professor Touyz’s lab. “The experiences I had in these labs motivated me to pursue my research in the field of vascular pathobiology,” she says.

Using the fellowship, Dr Briones spent 12 months working with Professor Touyz on the role of different subunits of vascular NADPH oxidase in hypertension, and she worked on a new project on the role of adipocytes and aldosterone in diabetes-associated obesity. Working on animal models and patient tissue, she found that adipocytes produce aldosterone that influences vascular function in diabetes. “My time in Ottawa was very productive,” says Dr Briones. “Not only was I exposed to a new field of vascular research, but I published several articles. I had the opportunity to initiate new collaborations, develop strong networks, and participate in many international scientific meetings.” After her return to Spain, Dr Briones won a Ramon y Cajal contract and joined the group of her PhD supervisors, Professor Mercedes Salaices, PhD, and Maria-Jesús Alonso, PhD, in Madrid.

Ester Macia, MD, consultant cardiologist and electrophysiologist, Arrhythmias and Electrophysiology Department, Capio-Fundacion Jimenez Diaz, Madrid

Dr Macia was awarded a Caja Madrid Foundation fellowship in 2008 to complete her training as an electrophysiologist by doing some basic research on arrhythmias. “It gave me the opportunity to work in an internationally recognised centre in preclinical research,” she says. The fellowship covered her living expenses, travel costs, and health insurance for 1 year of research in the group of Professor Penelope A. Boyden, PhD, in the Pharmacology Department, Columbia University Medical Center, New York, NY. The research focused on the arrhythmogenic substrate of the border zone of healing myocardial infarcts. “We studied the structural and functional remodeling of the ventricular gap junction protein, connexin43 in an animal model of myocardial infarction,” says Dr Macia. “In arrhythmogenic substrates connexin43 is a potential target for antiarrhythmic therapy. In this context, we examined the effects of the gap junction-specific agent, rotigaptide.” The research led to an article in Circulation: Arrhythmia and Electrophysiology. Dr Macia also had the opportunity to coauthor, with Professor Boyden, a Controversies in Cardiovascular Medicine article for Circulation titled “Stem Cell Therapy is Proarrhythmic.”

Dr Macia is currently supervised by Professor Jerónimo Farré, MD, PhD (see http://circ.ahajournals.org/content/119/2/f7). She says, “As a result of the experience acquired during my Caja Madrid fellowship, I am now participating in several clinical and translational research projects.”

Carlos G. Santos-Gallego, MD, postdoctoral fellow, Zena and Michael A. Weiner Cardiovascular Institute, Mount Sinai School of Medicine, New York, NY

At the Zena and Michael A. Weiner Cardiovascular Institute, Dr Santos-Gallego is supervised by Professor Juan J. Badimón, PhD, director of the Atherothrombosis Research Unit, and Valentín Fuster, MD, PhD (see http://circ.ahajournals.org/content/115/13/f55), director of the Cardiovascular Institute. “The work being developed in Professor Badimón’s lab on the pathogenesis of atherothrombosis and ischaemia-reperfusion injury and diagnosis by noninvasive imaging techniques was the ideal environment for developing my own career,” says Dr Santos-Gallego. “It perfectly suited my own interests, and it has offered me the possibility of broadening my scientific perspective and learning cutting-edge techniques for my development as a physician-scientist.”
Dr Santos-Gallego’s current research interests focus on the evaluation of cardiac function by noninvasive imaging (magnetic resonance and 3-dimensional echocardiography), pharmacological strategies for cardioprotection (using a porcine closed-chest model of ischaemia-reperfusion) and atherosclerosis (especially the role of high-density lipoprotein cholesterol).

During medical school, Dr Santos-Gallego studied the role of transforming growth factor-beta in atherosclerosis under the supervision of Professor Teresa Tejerina, MD, PhD. He says, “My first article described the important role of transforming growth factor-beta in the aspirin-induced inhibition of plaque proliferation; it was published in Circulation so we were very excited.” He then completed his cardiology fellowship at Fundación Jiménez Díaz Hospital in Madrid, under the supervision of Professor Farré. He received the 2009 SANITAS Award for the best medical fellow in all Spain endorsed by the Spanish Ministries of Health and Education.

Georgia Sarquella-Brugada, MD, PhD, research fellow, Interventional Cardiology, Electrophysiology and Cardiac Genetics, Paediatric Cardiology Service, Hospital Sant Joan de Déu for Children, University of Barcelona, Barcelona, Spain

Dr Sarquella-Brugada used a 12-month Caja Madrid Foundation fellowship awarded in 2007 which covered her expenses and university fees to conduct research at the Centre of Genetics and Congenital Heart Disease, Sainte-Justine Hospital, Montréal, Canada.

After training in paediatric cardiology, Dr Sarquella-Brugada’s subspecialty training was on interventional techniques and the genetic background of congenital heart disease. She carried out genetic screening of families with left ventricle outflow tract obstruction (in all their categories). Her previous articles were an important contributing factor to gaining the fellowship, as was the proposed training programme that would be supported. “This funding allowed me to do my subspecialty training and to recruit and genetically screen several families,” says Dr Sarquella-Brugada. “Now we are doing a whole genome scan of these families to find the genetic basis for their diseases.”

Dr Sarquella-Brugada has now moved back to her home town of Barcelona to continue her career and has maintained collaboration with the Canadian group in Sainte Justine to finish the project.

Susanna Prat González, MD, consultant cardiologist, Thorax Institute, Hospital Clinic, Barcelona

Dr Prat used a 2-year Caja Madrid Foundation fellowship awarded in 2006 to study the role of beta blockers in acute myocardial infarction at Mount Sinai Heart Hospital, New York, NY, from November 2006 to November 2008. Previously, she had completed her cardiology fellowship in Barcelona and conducted basic science research on cardiac stem cells in Kyoto, Japan from 2004 to 2005. She says, “It was a really good fellowship, providing a monthly salary, medical and travel insurance, and a round trip ticket.”

During the fellowship Dr Prat collaborated with Dr Borja Ibañez, MD (see http://circ.ahajournals.org/content/120/1/f1 and shown in the photo above with Dr Prat in the cardiac magnetic resonance imaging facility of Mount Sinai Medical Center), who at that time was a research fellow with Professor J. J. Badimón. They studied the cardioprotective effects of the β-1 selective blocker metoprolol in a swine model of acute myocardial infarction using cardiac magnetic resonance and they found that the administration of metoprolol during ongoing ischaemia was extremely cardioprotective compared with placebo. Their results were published in Circulation in 2007.

Dr Prat continues with noninvasive imaging techniques such as echocardiography, cardiac magnetic resonance, and coronary computed tomography at Hospital Clinic in Barcelona. She says, “I am happy with and thankful for the Caja Madrid fellowship because my experience in New York has been very useful for my medical and scientific career.”

Jennifer Taylor is a freelance medical journalist.

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