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My Continuing Evolution as a Surgeon-Scientist: A Decade after the Jacobson Promising Investigator Award

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The Second Joan L and Julius H Jacobson Promising Investigator Awardee, Edith Tzeng MD, FACS

In 2005, the Surgical Research Committee (SRC) of the American College of Surgeons was tasked with selecting the recipient of a newly established award, “The Joan L and Julius H Jacobson Promising Investigator Award.” According to the Jacobsons, the award funded by Dr Jacobson should be given at least once every 2 years to a surgeon investigator at “the tipping point,” who can demonstrate that his/her research shows the promise of leading to a significant contribution to the practice of surgery and patient safety.

Every year, the SRC receives many excellent nominations and has the difficult task of selecting one awardee. The first awardee was Michael Longaker MD, FACS who 10 years later reflected on the award and the impact it had on his career (1). This year, Edith Tzeng, MD, FACS the second Jacobson awardee reflects on her 10 year journey after receiving the award. Dr Tzeng is now a national and international figure in the field of vascular surgery and has studied the effect of nitric oxide and carbon monoxide on intimal hyperplasia.

Kamal MF Itani, MD, FACS and Leigh Neumayer, MD, FACS, on behalf of the Surgical Research Committee of the American College of Surgeons

I am extremely proud to have been the second recipient of the Jacobson Promising Investigator Award in 2006, a very humbling honor given the marked success and achievements of the other recipients of this Award. In the 10 years that have passed since I received this great honor, I have had a singular goal – to be committed to translational vascular surgery research in a way that promotes the joy and fulfillment of being a surgeon-scientist to future academic surgeons progressing through our training programs. My journey over the past 10 years is summarized in this report.

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For background, I completed my medical school education at the University of Chicago and then a general surgery residency at the University of Pittsburgh. I was instantly attracted to this training program because of the Chairman of Surgery at that time, Dr. Richard L. Simmons. His reputation for training academic leaders in surgery and successful surgeon-scientists is remarkable and was the true draw to the program. During my surgery residency, I also completed a 4 year post-doctoral research fellowship under Dr. Simmons' mentorship. I developed a great love for vascular surgery during residency due to the medical complexity of the patients, the lifelong relationships that are forged with these patients, the technical aspects of vascular reconstruction, and the enormity of the innovations in the field. I completed a vascular surgery fellowship at the University of Pittsburgh in 2000 and stayed on as faculty. As a surgeon-scientist, I have focused on a line of investigation that merges exceptionally well with my clinical expertise in vascular disease. During times of self-assessment, I find myself gravitating toward translational studies that allow me to marry laboratory findings to application in clinical disease, taking full advantage of my knowledge of vascular pathology and how disease is cared for clinically to adapt promising bench findings into practical therapies.

Nitric oxide synthase gene transfer in vascular disease

My interest in basic research dates back to my undergraduate education where I learned to construct retroviral mutants using recombinant DNA skills and was able to identify the mutation in the retroviral genome responsible for mediating disease (2). These skills served me well in my post-doctoral research training where I focused on developing therapeutic applications for the human inducible nitric oxide synthase (iNOS) cDNA. The early 1990s was the period when NO research exploded and Dr. David Geller and Dr. Timothy Billiar, both products of Dr. Simmons' mentorship, were the first investigators to clone the human iNOS gene (3). The importance of NO as a signaling molecule in the cardiovascular system was one of the earliest observations made by Dr. Robert Furchgott (4) who was one of three scientists to be awarded the Nobel Prize for discoveries in NO biology (5). Because of the ability of NO to inhibit smooth muscle cell proliferation and enhance endothelial regeneration/survival (6,7), a vascular application for iNOS gene transfer was an obvious first target with a focus on the prevention of intimal hyperplasia (IH) or restenosis in the setting of therapeutic vascular injury. Restenosis within stents and bypasses greatly impacts the patency of vascular interventions. By constructing viral vectors that carry the human iNOS cDNA, we demonstrated that expression of iNOS at sites of surgical vascular injury as well as immunologic injury (i.e., transplantation vasculopathy) markedly inhibited IH in both rodent and pig models (6,8–10) and even in the setting of enhanced inflammation of metabolic syndrome (11). We were also able to overexpress and produce quantities of iNOS protein that facilitated enzymology studies that provided mechanistic understanding of the assembly of inactive iNOS monomers into an active dimeric protein (12), findings that were instrumental in understanding and harnessing iNOS for clinical application.

These findings allowed us to obtain NIH funding to develop iNOS gene therapy to improve vascular graft patency with an initial target being the arteriovenous graft (AVG) that is placed for hemodialysis access. These AVGs are plagued with high failure rates due to accelerated neointima formation at the venous anastomosis secondary to flow dynamics and

compliance mismatch. The AVG provided an excellent target that permits local iNOS gene transfer with minimal systemic risk. Through this work, I gained valuable knowledge of the Recombinant DNA Advisory Committee approval process as well as the preparatory phases of an Investigational New Drug application to the FDA. While this clinical trial never came to fruition due to instability of the iNOS gene in the adenoviral vector that prevented production of clinical grade virus, I gained a great deal of knowledge in clinical trial design and the FDA regulatory processes.

Carbon monoxide as a vascular therapy

Another small gas molecule that bears great similarity to NO in function is carbon monoxide (CO). CO is best known as a silent killer, resulting in asphyxiation due to its 200 fold greater affinity for hemoglobin than oxygen (13). While it is an environmental toxin produced during combustion, it is also generated endogenously by heme oxygenase (HO) and possesses impressive anti-inflammatory and vasoprotective properties (14). HO metabolizes heme to the endproducts of biliverdin, free iron, and CO. The inducible isoform, HO-1, is expressed during times of organ or tissue injury and limits inflammation and is protective to the tissues (14). The anti-inflammatory actions of HO-1 are mediated by CO as well as biliverdin. In collaboration with Dr. Augustine Choi and Dr. Leo Otterbein, both experts in HO-1 and CO biology, we demonstrated that a brief treatment with inhaled CO at a very safe dose of 250 parts per million markedly reduced angioplasty mediated neointima formation in rodents (15). Inhaled CO also inhibited transplantation vasculopathy (15) and reversed cardiac and arterial remodeling in pulmonary hypertension (16). Perioperative inhaled CO also mediated vasoprotection in a porcine model of angioplasty injury without any signs of toxicity (17). Ex vivo CO also inhibited vein graft stenosis in rodents (18). Outside of the vasculature, inhaled CO has been shown to protect against hemorrhagic shock (19), necrotizing enterocolitis (20), and sepsis (21) making it a very attractive and potentially very effective systemic therapy. The mechanisms by which inhaled CO mediates vasoprotection continue to be an active area of investigation in my laboratory (15, 22, 23). Our studies indicate that inhaled CO induces changes that cannot be reproduced by directly treating cells with CO. Understanding these systemic mechanisms of action will allow us to harness and evolve inhaled CO into a therapy for vascular disease and other inflammatory disorders.

Coming full circle: back to NO

One of the limitations of NO based therapy that has complicated the translation of NO to clinical application is its very short half-life and reactive nature that make targeted delivery difficult. Although it shares great similarity to CO, it does not have the same therapeutic effectiveness when delivered inhalationally (24). In the last several years, however, an alternate pathway for NO production has been identified in which its breakdown products of nitrite and nitrate are converted back to NO through the actions of oral bacteria and tissue nitrite reductases (25). The cardiovascular protective effects of the Mediterranean diet have been linked to the consumption of food rich in nitrites and nitrates that feed into this alternative pathway (26). In collaboration with Dr. Mark Gladwin, one of the first investigators to describe this pathway, we reported that sodium nitrite delivered via oral, intraperitoneal, or inhalational routes can dramatically inhibit vascular injury induced

neointima formation in rodents (27). This protective effect was mediated by xanthine oxidoreductase (XOR), an enzyme that possesses nitrite reductase activity and is induced in the vascular wall following injury (27). Similarly, work by my former trainee and current collaborator, Dr. Brian Zuckerbraun, showed that dietary nitrite reversed the vascular and cardiac changes of established pulmonary hypertension (28). These findings were extremely important because they demonstrate the ability to generate vasoprotective effects using a very inexpensive and safe therapy of dietary sodium nitrite. This concept is being carried forward in nutritional supplements based on nitrite/nitrate therapy to improve cardiovascular health (29).

As a vascular surgeon, one of the very frustrating problems we treat is the nonhealing diabetic foot wound. We have previously shown that mice deficient in endothelial NOS experience impaired wound healing and restoring wound NOS activity returned healing to normal (30). We examined skin and wound tissue from mice for XOR expression and found high levels of expression in the skin and wound edge (31). We have also shown XOR in human wound tissues. XOR metabolizes purines and generates reactive oxygen species (ROS) in the process. Our studies indicated that XOR is necessary for normal wound healing, potentially through the production of low levels of ROS but may also be related to the production of NO from nitrite. We have preliminary data that show XOR expression in wounds from diabetic mice and that nitrite therapy can accelerate wound repair in these mice. My collaborator, Dr. Gregory Kato, conducted a Phase I clinical study at the NIH which showed that topical nitrite therapy in patients with nonhealing sickle cell ulcers was safe and suggested improved wound healing and alleviation of pain (32). We are extremely encouraged that topical nitrite therapy may improve diabetic wound healing and are developing a clinical trial to test this. Such a therapy could provide a safe and cost effective treatment for a disease that is plagued with very costly treatments.

Mentorship

One of the most valuable aspects of my career has been mentorship. As the stresses of healthcare reform and diminishing reimbursements result in budget cuts in hospitals and departments of surgery, supporting a developing surgeon-scientist becomes a heavy investment that a lot of departments no longer wish to make. Similarly, the extremely competitive funding environment of the NIH may also be a deterrent for those considering academic careers. It is especially important in these current times that we mentor promising trainees toward academic careers. I take great pride in the Vascular Surgery NIH T32 training program that we have established at the University of Pittsburgh that focuses on developing vascular surgery clinician-investigators. As I was mentored by nationally renowned surgeon-scientists, namely Richard Simmons (my first Chairman) and Timothy Billiar (my current Chairman), it is only natural that I wish to carry on that tradition even if only at a fraction of the success achieved by them. Some of my proudest achievements have been receiving mentorship awards from the general surgery and vascular surgery trainees in our program. The clinical mentorship and support I have received and continue to receive from my Division Chief, Michel Makaroun, are also greatly appreciated and allows me to balance a very fulfilling clinical vascular surgery practice and my research career. Finally, the work

described in this article was the product of numerous past and present collaborators and trainees to whom I will always be indebted.

In closing, I am thankful for the vision and generosity that Dr. Julius H. and Mrs. Joan L. Jacobson had when they established the Jacobson Promising Investigator Award. It provided funds and, more importantly, the belief in my potential at an early stage in my faculty appointment that has helped me to persevere in my career. For this, I express my deepest gratitude.

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