From Neurovascular Laboratory to Clinic: A Journey Through Time

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Approximately 795,000 people experience a new or recurrent stroke each year. Mortality data from 2008 indicate that stroke accounted for about 1 of every 18 deaths in the United States (US). On average, someone in the US has a stroke every 40 seconds [Veronique LR et al. *Circulation* 2012]. Gregory J. del Zoppo, MD, MS, FAHA, University of Washington, Seattle, Washington, USA, discussed changes in how we understand brain microvessel structure, neuron-vascular interactions, and the translation of scientific findings into clinical care in The Thomas Willis Lecture, entitled "Toward the 'Neurovascular Unit:' A Journey in Clinical Translation."

In the mid- to late-1970s, the therapeutic attitude toward stroke patients was nihilistic, with few specific treatment approaches, CT scanning in its early days, and little data on the risks and benefits of antithrombotic approaches. Fibrinolysis was contraindicated, and cerebral blood vessels were considered inert conduits.

Since then, experimental and clinical data have increased our comprehension of the molecular processes that cause ischemic damage to neurons and glia, the simultaneous mechanisms that underlie the maturation of the ischemic lesion to infarction, and the acute responses of microvessels in the ischemic territory. However, there is a need to understand these processes [del Zoppo GJ. *J Intern Med* 2010] as a single neurovascular unit (ie, to link microvessel with neuron [neuron-vascular] interactions) [del Zoppo GJ. *J Intern Med* 2010]. Dr. del Zoppo's presentation detailed the work over 3 decades that has defined the impact of ischemia during stroke on microvessel function and responses in the context of neuronal damage.

The Neurovascular Unit

The "neurovascular unit" consists of microvessels, astrocytes, neurons and their axons, and other supporting cells that are likely to modulate the function of the "unit" [del Zoppo GJ. *Ann NY Acad Sci* 2010]. It is a conceptual framework that links microvessel and neuron function and their responses to injury. It is also a structural arrangement that links microvessel components with neurons via common astrocytes.

In the central nervous system, microvessel-neuron interactions appear to be highly coordinated. In experimental ischemic stroke, the rapid simultaneous reactions of the microvasculature, neurons, and glia to focal ischemia suggest that these responses could be viewed in a unitary fashion rather than as individual components [del Zoppo GJ. *Ann NY Acad Sci* 2010].

Neurovascular coupling enables neurons to modulate cerebral blood flow in regions of activation. Although little is known about the microvessel-to-neuron direction of the relationship, data support a unitary hypothesis in which microvessel and neuron responses are related, with implications for signaling events, potential treatment targets, and clinical outcomes.

The Journey to the Neurovascular Unit

Dr. del Zoppo discussed a wide range of new findings that have been studied *en route* to the notion of the neurovascular unit. These include the variety and rapidity of intravascular events following ischemic stroke, in particular endothelial cell responses to ischemia, the focal "no-reflow" phenomenon and its relation to polymorphonuclear (PMN) leukocyte adhesion, fibrin formation, platelet activation, and tissue factor expression (Table 1);

matrix responses to focal ischemia and their relationship to pro-MMP-2, urokinase (u-PA), and neuron injury; and plasminogen activator expression by microvessels.

Table 1. Focal "No-Flow" Phenomenon.

Element	Event	Effect
PMN leukocytes	Activation	Adherence/obstruction
Fibrinogen	Fibrin formation	Deposition/obstruction
Platelets	Activation	Thrombosis/obstruction

The generation of matrix proteases during ischemia is rapid, as are changes in endothelial cell adhesion and adhesion of astrocyte end-feet. These changes involve loss of the matrix adhesion receptors αβ-dystroglycan and integrin α6β4 on astrocytes and β1-integrins on endothelial cells. They occur following middle cerebral artery occlusion in a large animal model of focal ischemia and can be reproduced in cell culture. The microvessel basal lamina is degraded in the ischemic core, and astrocytes detach. An important recent finding is that the endothelial cell permeability barrier opens at the same time that adhesion receptor expression changes. The loss of β1-integrins from endothelium leads to disruption of tight junction proteins that form the endothelial cell barrier and increased permeability.

The Global Search for Solutions

Early acute intervention studies focused on the feasibility and safety of plasminogen activator infusions, intracerebral hemorrhage risk control, and potential improvements with specific agents, including urokinase and tissue plasminogen activator. In the laboratory, the del Zoppo group has used focal ischemia as a "wedge" to elucidate interactions of microvessels with the neurons they serve. Findings are based on precise clinical observations of acute events in the microvasculature in a high-quality, nonhuman primate focal ischemia model, with direct application to patients in clinical trials.

Early investigations centered on whether decreased patency of capillaries could be prevented or reversed; whether adherence of leukocytes—as well as fibrin formation, platelet activation, and peripheral inflammation—could be blocked; and whether the "no-reflow phenomenon" could be prevented.

If no-reflow could be prevented, and fibrin formation, platelet activation, and adherence of leukocytes that occlude microvessel adhesion receptors could all be blocked, patency would be increased and preserved. "We all want to stop or reverse ischemic cerebral tissue injury, but the transition of knowledge from the lab to the clinical environment is an inexact art," Dr. del Zoppo said, adding that trials have not necessarily supported changes in clinical care. "Understanding why at the level of mechanisms of tissue injury is essential to correct our course."

The Need to Know

Endothelial cells facilitate cellular inflammation and rapidly generate and respond to proinflammatory stimuli following ischemia onset. But how individual cells within the unit communicate with each other during postischemic inflammatory responses is not clear: a unifying synthesis of inflammatory responses has not yet been developed [del Zoppo GJ. *J Intern Med* 2010].

In microvessel-neuron relationships, there are region-specific arrangements of the microvasculature. This is in agreement with differences in regional cerebral blood flow, whereby flow is lowest in the corpus striatum and highest in the cortical grey matter. The relative arrangements and densities of the neurons (and neuron subtypes) to the 'supply' microvessels in these regions are not known [del Zoppo GJ. *J Intern Med* 2010].

The coordinated and potentially unitary nature of microvessel-neuron relationships in the acute setting of focal ischemia is suggested by known matrix responses to focal ischemia. As a framework for understanding the responses to acute intervention during ischemic strokes, further basic information is required that adequately relates microvessel reactivity and changes in neuron integrity in mammalian systems [del Zoppo GJ. *J Intern Med* 2010].

The Way Ahead

Dr. del Zoppo observed that the proper acute delivery of plasminogen activators is very effective. Microvessel responses are as rapid as those of neurons and include endothelial cell activation, altered matrix architecture, and decreased adhesion receptor expression. He noted that the acute appearance of proteases with matrix-degrading capability and the events in microvessels are heterogeneously distributed. The responses of the endothelium, the permeability barrier and tight junction proteins, astrocytes, and their matrix adhesion are all connected in time and space. Dr. del Zoppo cited the need to identify the cellular sources of proteases that are acutely related to microvessel responses and regions of neuronal injury.

These observations suggest that great care in the translation from experimental studies to Phase 1/2 clinical safety assessments and the design and conduct of Phase 3 trials is needed [del Zoppo GJ. *Ann NY Acad Sci* 2010].