## 2005 Mihara Prize Awardee' s Summary

### CELL THERAPY AND MICROVASCULAR CHANGES IN CEREBRAL ISCHAEMIA : *in vivo* IMAGING STUDIES

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Due to his double specialization in Physics and Neurobiology, Jacques Seylaz has, all along his career, focused his research activity on two distinct domains: bioengineering and cerebrovascular physiology and pathophysiology. He both developed new methods to investigate brain blood flow and metabolism in animals and humans, and applied these methods to explore new concepts in cerebrovascular physiology and pathophysiology. From the outset, he postulated that intracerebral pathways were involved in the control of cerebral blood flow (CBF). The overall objective of his research was always to improve diagnostic tools and therapeutic protocols for patients with cerebrovascular diseases.

Jacques Seylaz created the first French laboratory devoted to experimental research on CBF. First, he began by developing a thermoclearance method for semi-quantitative continuous measurement of local CBF. He progressively elaborated a monitoring of several cerebrovascular variables in humans that was mainly used during carotid surgery. At first he investigated CBF changes during various phases of sleep, demonstrating by continuous measurements that CBF increases during slow wave sleep and paradoxical sleep. In opposition to the prevailing theory at that time, his data demonstrated that the electroencephalogram cannot be used as an index of CBF. In addition, he determined that atheromatous disease is linked to dysregulation of cerebrovascular reactivity, and established criteria to diagnose the pathology on the basis of CBF changes measurements, in hypercapnic conditions for instance.

In parallel, Jacques Seylaz developed an original method of non-invasive measurement of regional brain blood flow using 133Xenon that led to the production and the marketing of an automated apparatus. His data provided several pathophysiological mechanisms of local vascular insufficiency following Sylvian artery occlusion, and

facilitated surgical decisions such as in the case of the blood supply surgery in case of temporo-sylvian anastomoses, or liquid derivation in case of normal pressure hydrocephaly.

Jacques Seylaz contributed to devise a research strategy by developing various complementary approaches whose main objectives were to define the functions of cerebral blood vessels innervation, the regulatory mechanisms of CBF, and the relationships between brain blood flow, metabolism and lesions in some cerebrovascular diseases.

Local metabolism was known to play a significant role in CBF control, but the precise mechanisms were not known, as well as the balancing part of the nervous system. To investigate their respective roles in depth, Jacques Seylaz adapted mass spectrometry to continuously measure PO2 and PCO2 in brain tissue simultaneously, thus providing a direct quantitative evaluation of local oxidative mechanism. Taking advantage of this method, he developed Helium clearance for simultaneous quantitative determination of local blood flow. An apparatus for routine use was then developed.

He also led studies aiming at determining the mechanisms of cerebral autoregulation, the causes of loss of consciousness under high gravity and also the mechanisms involved in changes in CBF during an immobilization stress.

Progressively, he managed to combine these techniques with quantitative autoradiography and nuclear magnetic resonance (NMR). He oriented his team towards the search of a vascular influence of the autonomic nervous system on CBF, using stimulation and denervation strategies and combining histological and functional approaches. With his research team, he demonstrated that CBF is under a neurogenic control. In collaboration they notably established the influence of sympathetic, parasympathetic and trigeminal innervations of cerebral vessels, and the vasomotor role of catecholamines. They also demonstrated that the activation of entirely intracerebral nerve fibers locally modifies CBF. When nitric oxide (NO)-releasing nerve fibers were identified along cerebral vessels, he initiated immunohistochemical studies using fluorescence confocal microscopy, showing co-localization of NO-fibers from interneurons and intracerebral microvessels. His team also worked on the various conditions in which neuronally-derived NO exerted its vasodilatory influence.

He studied local CBF control under activation stimuli such as in epileptic seizures. His team contributed to establish the role of adenosine and NO released from neurons in local microcirculatory changes under these pathophysiological conditions.

Jacques Seylaz also enlarged the research work by introducing several ischemia models to determine the role of glutamate in the ischemic lesion. Using NMR imaging and

spectrometry, microdialysis, and histological evaluation of the lesion gravity in rodents, his team contributed to the characterization of the ischemic cascade and to the demonstration of the neuroprotective role of magnesium, the natural blocker of the NMDA receptor-associated channel.

The latest method he developed aimed at investigating in vivo and in real-time the cortical microcirculation of rats and mice. He used laser scanning confocal fluorescence microscopy to videomonitor fluorescently labelled red blood cells through a closed cranial window. This method enables the dynamic monitoring of changes in capillary circulation at 200-µm-deep in the cortex of animals placed under a microscope. In rats, this method was used to explore red blood cells flow through capillaries during either peri-ischemic cortical spreading depression or hyperemic reactions to transient global ischemia, to relate them to microvessel diameter changes and to reversal of blood flow, and to evidence the role of NO released by neurons in increases in local microcirculation.

In mice, he first elaborated a cranial window that can be used chronically, i.e. for repeated imaging sequences over at least 1 month. Then, to enable the study of microcirculation within the core of ischemia, Jacques Seylaz designed a new strategy using mice by thermocoagulating distal branches of the middle cerebral artery directly through the cranial window. The characterization of this new model was performed in vivo through the chronic cranial window, and using magnetic resonance imaging. A complementary characterization was performed ex vivo by immunohistochemistry.

Finally, in an innovating way, he is currently conducting an investigation of the potentiality of cell therapy in the brain, by monitoring the in vivo fate of various stem cells transplanted into the mouse ischemic brain. The whole strategy is based on the in vivo use of laser-scanning confocal fluorescence microscopy through a cranial window to visualize transplanted cells in the long term. These cells are harvested in transgenic mice overexpressing the green fluorescent protein (GFP) and stereotaxically injected into the cortex of mice with focal cerebral ischemia. Globally, the data of his team demonstrate that stem cells of various origins migrate to the ischemic and periischemic areas and could be used as vehicles to introduce therapeutic genes into the nervous system to induce a beneficial effect on brain recovery.

This approach will constitute the bases of his research program for the coming years.

Jacques SEYLAZ has been the head of a French State Laboratory funded by CNRS (National Center of Scientific Research), by INSERM (National Institute of Health and Medical Research) and by the University of Paris VII. He has been involved in other Research Programs in France in various capacities. He was a member and then chair of

National Committees of CNRS and chair of Life Sciences of CNES (National Center for Space Studies). He was Deputy Director for Life Sciences at CNRS and a member of the French CCNE (National Consultative Ethics Committee).